“LIQUID BASED CYTOLOGY OVER CONVENTIONAL PAP STAINING METHOD IN EVALUATING CERVICAL SMEARS”

DISSERTATION SUBMITTED FOR

M.D. DEGREE EXAMINATION

BRANCH III PATHOLOGY

OF

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

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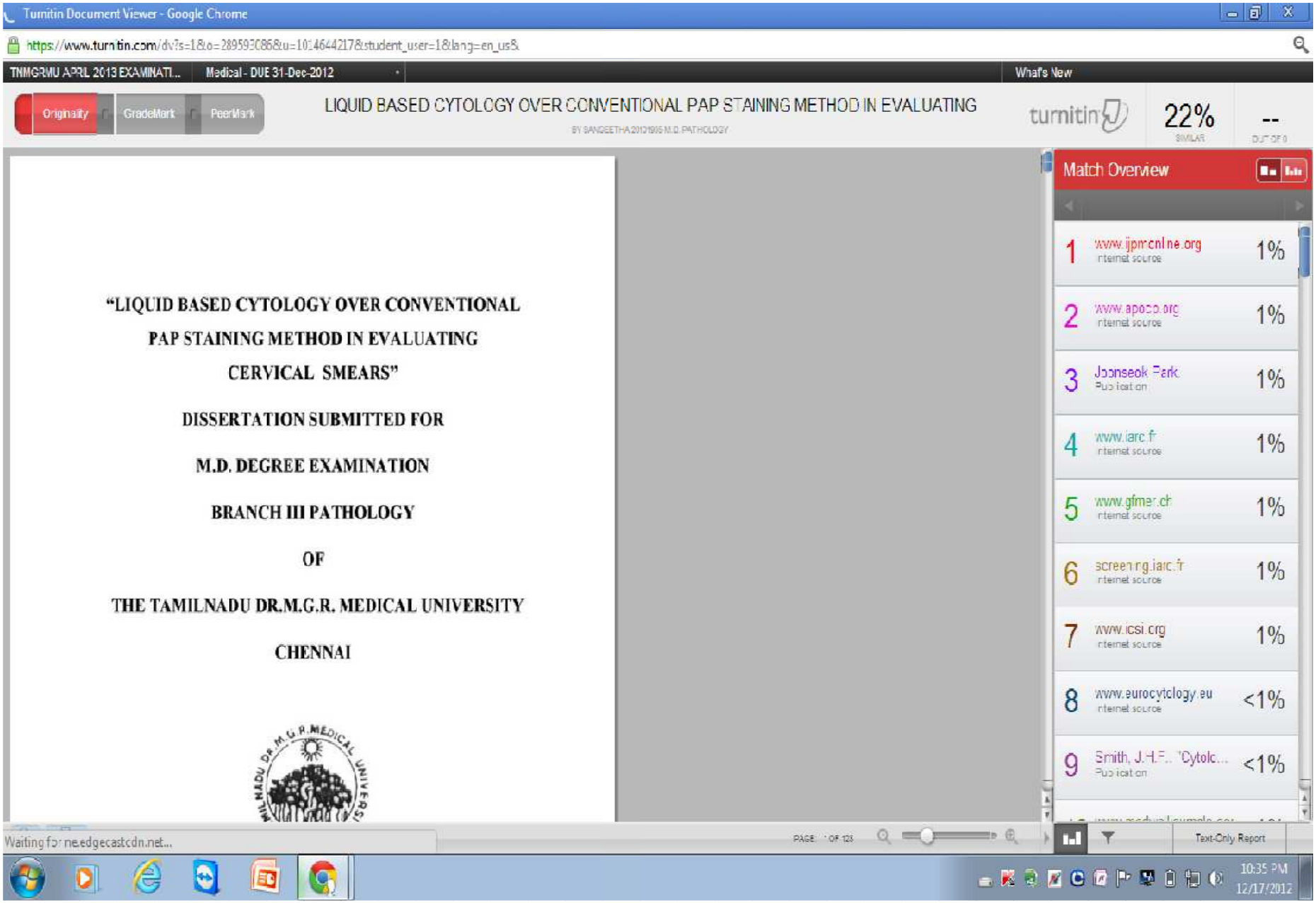
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I hereby certify that this work embodied in the dissertation entitled "**LIQUID BASED CYTOLOGY OVER CONVENTIONAL PAP STAINING METHOD IN EVALUATING CERVICAL SMEARS**" is a record of work done by **Dr. A. SANGEETHA,** in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during her postgraduate degree course in the period 2010-2013. This work has not formed the basis for any previous award of any degree.

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DECLARATION

I solemnly declare that the dissertation titled "**Liquid Based Cytology Over Conventional Pap Staining method In Evaluating Cervical Smears**" is done by me at Tirunelveli Medical College hospital, Tirunelveli.

The dissertation is submitted to The Tamilnadu Dr. M.G.R.Medical University towards the partial fulfillment of requirements for the award of M.D. Degree (Branch III) in Pathology.

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CONTENTS

ABBREVIATIONS

LBC	:	Liquid Based cytology
MLBC	:	Manual Liquid based cytology
CS	:	Conventional smear
LP	:	LiquiPrep
CIN	:	Cervical intraepithelial neoplasia
NILM	:	Negative for intraepithelial lesion or malignancy
NILM-IS	:	Negative for intraepithelial lesion or Malignancy - inflammatory smear
ASC	:	Atypical squamous cells
ASCUS	:	Atypical squamous cells of undetermined significance
AGC	:	Atypical glandular cell
ASC-H	:	Atypical squamous cells-cannot exclude HSIL.
LSIL	:	Low grade squamous intraepithelial lesions
HSIL	:	High grade squamous intraepithelial lesions
SCC	:	Squamous cell carcinoma
HPV	:	Human papilloma virus

INTRODUCTION

Cervical cancer is the third most common cancer among women worldwide & second most common cancer in developing countries, over half of which are fatal (Jemal A et al 2011)[1]. About 80% of these cases and deaths occurred in developing countries (Ferlay et al 2010)[2].This high mortality makes cervical cancer an important public health problem. Cervical cancer is slow growing and hence has potential for effective prevention through various screening procedures. Cervical cytology has proved to be one of the most successful examples of cancer screening in many developed countries and has resulted in significant decrease in incidence and mortality from invasive cancer by detecting and eradicating the pre invasive lesions (Clarke EA 1979)[3](Hakama M et al 1985)[4](Miller AB et al 1990)[5] (Mathew A et al 2009)[6].

Invasive cervical cancer is the end result of a long pathological process that begins with precursor lesion called squamous intraepithelial lesions .Early changes in the cervix in the form of CIN can be detected years before invasive carcinoma develops and this is the basis of effectiveness of cytological screening (Wright T et al 1994)[7]. The main objective of cervical screening is to decrease worldwide incidence and mortality of cervical cancer, by detecting and treating precancerous lesions.

The screening of cervical cytology smears was introduced in 1928 by Dr.George N.Papanicolaou when he reported the observation of dysplastic/malignant cells in women with cervical cancer by sampling vaginal smears. Subsequently Papanicolaou and Dr.Herbert Traut identified cells of both invasive and pre invasive cervical neoplastic lesions by cervical cytology. This test is now known as the conventional Pap smear or Pap test (Papanicolaou et al 1941)[8].

For Conventional cervical cytology cell samples taken from the cervix by using Ayre's spatula is smeared onto slides, fixed and stained with Papanicolaou stain. Specificity of this test is 98 to 99%. But the sensitivity, ranges from 50%-75% (Fahey MT et al 1995)[9](Nanda K et al 2000)[10]. Several limitations of conventional pap test are identified such as 1) Inadequate transfer of cells to slide, 2) Un uniform distribution of abnormal cells, 3) Presence of obscuring inflammation, blood and overlapping of epithelial cells (Richart RM et al 1965)[11].

Liquid based thin layer technology was introduced as a FDA approved alternative method to conventional Pap in 1996 to address these limitations. The first generation automated Liquid-based cytology (LBC) involves rinsing the sampling device into a vial of fixative to form a suspension of cells from which a monolayer of cells on a slide is prepared. These slides can be read more quickly than Conventional smears & the residual sample can be used for HPV DNA testing. There

are two methods of FDA approved LBC technologies -ThinPrep and SurePath (Lee RL et al 1997)[12] (Monsonego J et al 2001)[13](Fang-Hui Zhao et al 2011)[14].These new LBC techniques require an automated instrument and so higher cost per test.

LiquiPrep, the second generation Liquid Based Cytology system eliminates most of the instruments required by the first generation techniques thereby offering a simpler method with lower costs for cervical cancer screening. LiquiPrep system consist of fixative fluid vial, a cleaning solution & a cell base that act as a membrane matrix to produce a monolayer of cells(Geyer J et al 2004)[15]. Easy method of preparation & high correlation of results obtained with CS makes this method highly suitable for cervical cytology in developing countries (Jongkolnee Settakorn et al 2008)[16].

There are a few studies showing an Indigenous method of Liquid based Cytology called Manual Liquid Based Cytology (MLBC). In this method chemicals available in their own laboratory were used to prepare fixative and polymer solution and use simple equipments to prepare cervical smear slides. This is a low cost method of cervical pap smear screening (Maksem et al 2001)[17] (Maksem et al 2005)[18] (Lee et al 2006)[19](Kavatkar et al 2008)[20](Nandini et al 2012) [21].

The present study was undertaken to compare this low cost Manual LBC with conventional pap cytology .In addition we also compared second generation LiquiPrep system with conventional smears.

AIMS AND OBJECTIVES

Aim of this study is

1. To evaluate the efficiency of a new inexpensive Manual Liquid Based Cytology.
2. To make a comparative morphological analysis of Conventional Papanicolaou stained cervical smear with Manual liquid based cytology smear.
3. To make a comparative morphological analysis of Conventional Papanicolaou stained cervical smear with Second generation LiquiPrep cytology smear.
4. To make a comparative analysis of the results of both the Liquid Based Cytology methods with Conventional smears.

REVIEW OF LITERATURE

CARCINOMA CERVIX

Epidemiology

Cervical cancer is one of the most common cancer among women worldwide (WHO 2009)[22].Majority of cervical cancer cases today occur in the developing countries. According to National Cancer Registry of ICMR the incidence in India is 14.42/100000 pop with mortality rate 2.83/100000 pop (ICMR 2004)[23]. Before the introduction of screening, the rates of cervical cancer in Europe, North America and Japan were very similar to those now seen in developing countries. Over past several decades the incidence rate has declined in both white and African American women. Since 2004, rates have decreased by 3.1% per year in women 50 and above (American Cancer Society, Cancer Facts & Figures 2012)[24].

Role of Human Papilloma Virus

Cervical cancer is unique among human cancers by being the first to be found almost completely attributable to the effects of an infectious agent(Thomison et al 2008)[25].The most important risk factor for cervical cancer is infection with a high-risk strain of human papilloma virus and persistence of HPV infection .For his discovery of HPV as a cause of cervical cancer , Harald Zur Hausen was awarded the noble prize in 2008.

More than 150 types of HPV exist. Of these, 15 are classified as high-risk types (Walboomers J.M et al 1999)[26] of which types 16 and 18 together with 31 contribute to 70% of cervical cancer cases (Munoz N et al 2003)[27]. HPV infection of the cervical epithelium is usually transient and produces cellular immune response that seems to be the most important factor for regression. Any event inhibiting normal differentiation of the epithelium or preventing normal sequence of viral replication may lead to the development of persistent infections, which can remain clinically latent or become active due to a compromised immune status or other factors. Progression of this HPV infection to cancer may be influenced by other factors including immune suppression, high parity, cigarette smoking and long term use of oral contraceptives (American Cancer Society,Cancer Facts & Figures 2012)[24].

HPV mainly infects immature metaplastic squamous cells present at squamo-columnar junction. But HPV replicates only in the maturing squamous cells & result in a cytopathic effect 'koilocytic atypia'. The koilocyte is a superficial or intermediate mature squamous cell characterized by densely stained peripheral cytoplasm and a large nuclei with an undulating nuclear membrane and a rope-like chromatin pattern with sharply outlined perinuclear vacuolation(Lee KR et al 1997)[28].HPV has to reactivate the mitotic cycle by interfering with the function of Rb and P53 through Viral E6 /E7 proteins to replicate in maturing

squamous cells . E7 viral protein binds with Rb gene and up regulates cyclin E, thereby promotes the cell cycle. Whereas E 6 protein binds to P53 and interrupt cell death (Schiffman M et al 2007)[29].

GROSS ANATOMY OF CERVIX

The cervix is the narrow inferior segment of the uterus which projects into the vaginal vault. It measures 3 cm in length and 2.5cm in diameter. The cervix is traversed by the endocervical canal .It has 3 parts.

1. The Endocervix lined by mucous secreting columnar epithelium.
2. The Ectocervix lined by non keratinising stratified squamous epithelium
3. The Squamocolumnar Junction (SCJ) - Due to metaplastic changes in the columnar lining of the cervix, the position of SCJ varies throughout the life. Before puberty the SCJ is usually located at the external os; in the parous women it lies on the ectocervix; after the menopause the SCJ is usually within the endocervical canal.

METAPLASTIC CHANGES IN THE CERVIX AND ITS PHYSIOLOGICAL BASIS

Exposure of endocervical epithelium to the acid pH of the vagina, act as a stimulus for metaplastic changes in the columnar epithelium.

The process of metaplasia starts initially in the crypts and at the tips of the endocervical glands. With progression entire endocervical

epithelium will be replaced by squamous epithelium .In the cervix, the area of the epithelium that has undergone metaplastic change is called the transformation zone (Fox H et al 1987)[30].

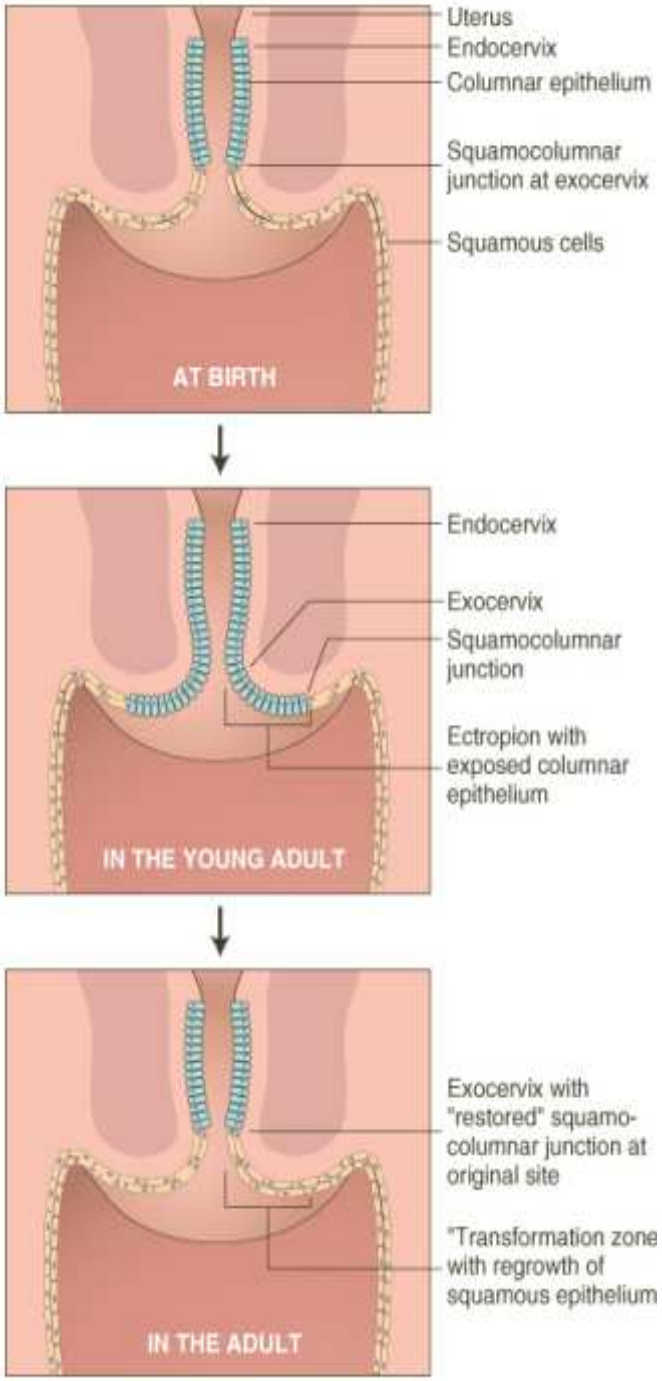


FIG 1: DYNAMIC ANATOMY OF CERVICAL EPITHELIUM

Three histological stages in metaplasia have been identified:

TABLE 1: HISTOLOGICAL CHANGES IN MEATPLASIA

STAGES	**CHANGES**
STAGE 1	Reserve cell hyperplasia
STAGE 2	Immature squamous metaplasia
STAGE 3	Mature squamous metaplasia

Numerous studies have shown that the immature metaplastic epithelial cells are susceptible to carcinogens and most, if not all cervical cancers arise here (Richart RM, 1973)[31]

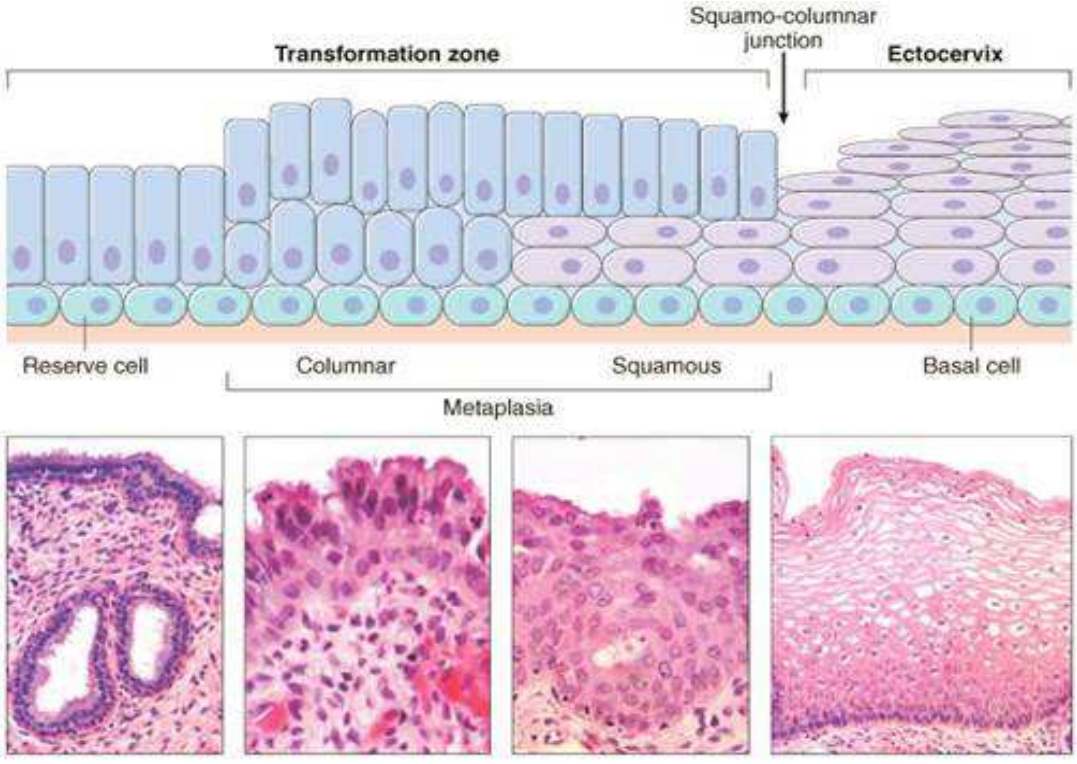


FIG 2: HISTOLOGICAL STAGES IN METAPLASIA

CERVICAL CYTOLOGY

The contents of cervical smears from a normal cervix is categorized as follows

1. **SQUAMOUS EPITHELIAL CELLS DERIVED FROM THE ECTOCERVIX**

The epithelial cells are classified based on cell size, shape, cytoplasmic staining property and N:C ratio. The commonly used Papanicolaou staining depends on pH , so the cytoplasm of superficial cells does not always take up eosin and the intermediate or parabasal cells is not always cyanophilic.

A. Superficial Cells

These are large angular cells measuring 50µm in diameter, shed from the surface layer of fully mature epithelium. They contain abundant cytoplasm that stains pink to orange with Papanicolaou stain and a single round pyknotic dark nucleus measures less than 5µm with a nuclear cytoplasmic ratio of 1:10(Boschaun H.W 1958)[32].

B. Intermediate Cells

These cells originate from middle layer of cervical epithelium. They are the most common cells seen in smears at post ovulatory time, during pregnancy, or as a result of action of progesterone. These are large angular cells measuring 30-50µm in diameter often with folding tendency of their cytoplasmic edge. They contain abundant cytoplasm that stains

blue to greenish blue with Papanicolaou stain and a single round vesicular nucleus measures 5-10µm with nuclear cytoplasmic ratio of 2:10. These cells usually appear in clumps.

During pregnancy these cells assume boat shape with thickened borders and eccentric nuclei due to effect of progesterone. These are referred to as Navicular cells. The cytolytic action of Doderline's bacillus on the fragile cytoplasm of intermediate cells forms many stripped nuclei (Bertalanffy F.D 1963)[33].

C. Parabasal /Basal Cells

Basal cells are the smallest cells seen in normal smear, they are commonly found in scrapped smears of an atrophic or deeply ulcerated mucosa. These are round to oval cells of 10-12µm size (about the size of leukocyte) seen in clusters. They have scanty deep blue cytoplasm and a round uniform nuclei with coarse chromatin , occupying one third of the volume of cytoplasm of the cell with N:C ratio of 8:10.Parabasal cells are similar to basal cells but the size range from 15-30 µm with blue cytoplasm and granular nuclear chromatin.

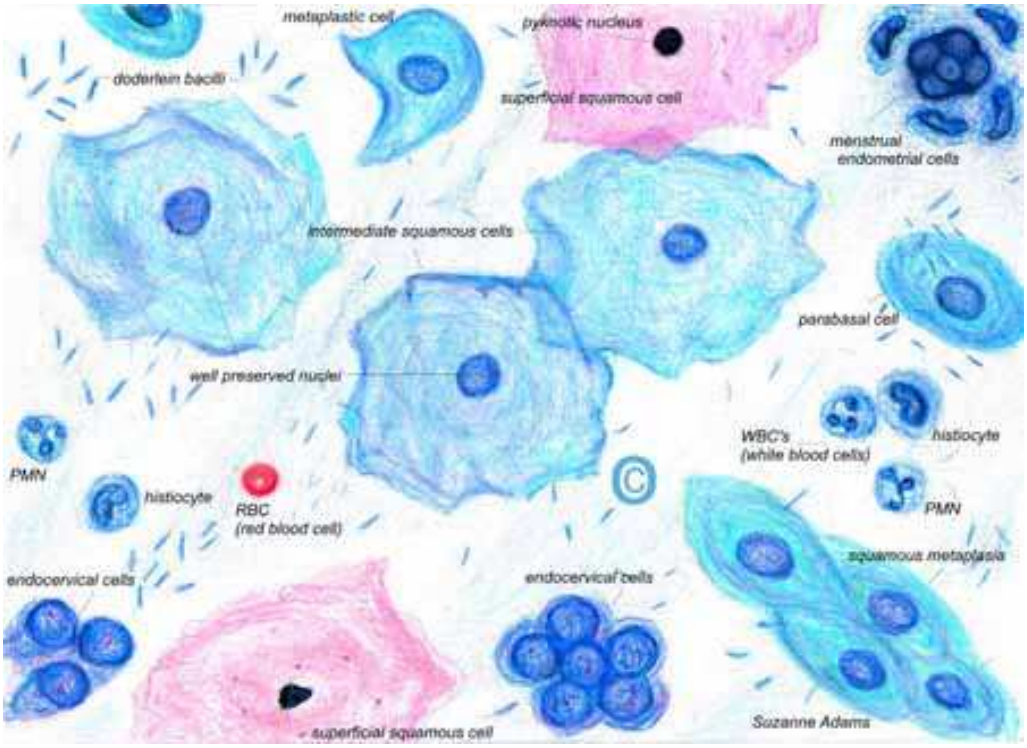


FIG 3: CONTENTS OF NORMAL CERVICAL CYTOLOGY

2. GLANDULAR EPITHELIAL CELLS FROM ENDOCERVICAL CANAL

These cells may appear single or in sheets (palisade or honeycomb appearance).They are uniform tall columnar cells of 10-25µm size with sharp smooth borders & abundant blue cytoplasm with occasional large cytoplasmic vacuoles. The nuclei are round to oval measuring 9-20µm in size with fine chromatin often with prominent nucleoli (Gondos B et al 1972)[34].

Sometime endocervical reserve cells can also be found .These are young endocervical parabasal cells capable of multipotential differentiation. They appear as sheets or clusters of oval cells measuring

8-20µm in size with scant cyanophilic , finely vacuolated cytoplasm and round to oval central nuclei with fine uniform chromatin, small nucleoli and occasional mitosis.

3. METAPLASTIC CELLS FROM TRANSFORMATION ZONE

A. Mature Squamous Metaplastic Cells

It resembles superficial or intermediate cell. These cells are usually found in sheets adjacent to normal endocervical cells. These cells are irregular in shape with well defined cell borders, abundant deep orange cytoplasm and an irregular nuclei with vesicular chromatin.

The presence of metaplasia increases the length of squamo columnar junction from which carcinoma arises. Metaplastic cells themselves are not considered to be a precursor of cancer (Fetherston W.C 1975)[35]. These cells must be differentiated from low grade invasive squamous cell carcinoma cells in which cytoplasmic keratosis & nuclear abnormalities are more.

B. Immature Squamous Metaplastic Cells

These cells resemble the parabasal cells found in atrophic smear. They are usually found in sheets and group. These are round to oval in shape that mould tightly against one another. The cytoplasm is dense, stains deep blue, pink or orange, with centrally located vesicular nucleus. These metaplastic cells are differentiated from parabasal cells in atrophic

smear by admixture of mature squamous epithelium. These cells are usually seen in smears of pregnant women and those on OCP.

4. CELLS FROM THE ENDOMETRIAL LINING AND STROMA

Endometrial cells may be found in normal cervical smears following menstruation, early pregnancy, postpartum period (Liu W et al 1963)[36]. They are found in tight clusters or in acinar pattern with a central core of stromal cells. These cells are smaller than basal cells (8-10µm), round to oval in shape with indistinct cell border. They have scant transparent green to pink cytoplasm and round uniform central nuclei having both fine and coarse chromatin resemble salt and pepper appearance. They differ from endocervical cells by regularity of size of nuclei, scantiness of cytoplasm, chromatin pattern and their exfoliation in tight clusters (Boschaun H.W 1958)[37].

5. COMMENSAL MICRO-ORGANISMS

Numerous organisms colonize in the vagina in the absence of disease. They include lactobacillus which appears as blue staining rod shaped organism 1-2µm in length, Diptheroids, Coliforms , Anaerobes and Enterococci.

6. OTHER COMPONENTS OF CERVICAL SMEARS

This includes leukocytes, erythrocytes, histiocytes, spermatozoa, and cervical mucus.

SCREENING METHODS

Screening is search for unrecognized malignancy by means of rapidly applied test to reduce the incidence and mortality from the disease. Early detection and timely treatment of early cancer & precancerous conditions provide the best protection against cancer. Objective of the National cervical Cancer screening program is to reduce the cervical cancer incidence and mortality by detecting and treating precancerous lesions. There are various screening procedures for cervical cancer that includes colposcopy, visual inspection, cervical cytology, cervicography and HPV testing.

Among these Cervical cytology is a widely used screening test in asymptomatic populations and in the follow-up of patients with cervical carcinomas treated by either conservative surgery or irradiation(Ducatman BS et al 2002)[38]. This can be done either by routine Pap test or by Liquid Based Cytology method.

1. CONVENTIONAL CERVICAL CYTOLOGY

Conventional cervical cytology involves taking samples from ectocervix & endocervix ,smearing onto glass slides, fixing and staining by Papanicolaou stain (Papanicolaou GN 1942)[39]. The Pap smear has been utilized for cervical screening for more than 50 years and has reduced the mortality rate of invasive cervical carcinoma by 50–70%

(Cramer DW 1974)[40]. In spite of this success, the Pap smear has a false negative rate reported as 55 %(Coppleson LW et al 1994)[41].

Errors are due to

1. Poor sampling and
2. Non representative samples
3. Inadequate transfer of the collected sample to the slides

The transfer of the collected material to the slide for smearing is usually done in clinic and so is subjected to many variations in the quality of fixation, amount of obscuring mucus, blood, inflammation, thickness of the smear and diagnostic homogeneity of the final preparation.

New generation of collection devices has greatly improved the sampling techniques as it dependably removes large and representative samples from the endocervix and ectocervix(Hutchinson M et al 1992)[42].

Careful attention to technical factors is essential to achieve good results. The smear should be promptly fixed and carefully stained. Air-dried smears are grossly inadequate in this regard. Even if squamous cells are rehydrated, they never exhibit the fine structural details of wet-fixed smears. The glandular cells are even more distorted.

Few studies have been done in the past to improve the cervical specimen cytology. A study by Steven et al (1997)[43] revealed that

chemical depolymerisation of cervical mucus helps to produce a monolayer sheets of cells.

2. LIQUID-BASED CERVICAL CYTOLOGY

Liquid-based cytology is an alternative to the conventional Papanicolaou (Pap) cytology smear for early detection of cervical abnormalities and cervical cancer. Liquid-based cytology tests purport to improve the quality of cervical specimens and increase the detection of cervical abnormalities (i.e. reduce the false-negative rate). With the conventional Pap test, a portion of the cell sample is lost when the sampling device is discarded and material such as blood and mucus may get on the slide and impede diagnosis (Gay JD et al 1985)[44](Goodman A et al 1996)[45]. Liquid-based cytology tests provides more representative portion of the cell sample and remove large portion of non diagnostic material. In this method samples are collected using a special cytobrush. The tip of the brush, which contains the sample is removed and placed into a vial containing a fixative to get more representative samples. In the laboratory blood, mucus and debris from the sample are also removed. The sample is then mixed to an even homogenous mixture that is placed on a glass slide to form monolayer sheets and stained with pap stain.

Liquid based methods add to the cost of a conventional Pap smear. However ,several studies showed that LBC method improved the quality of screening through improved specimen adequacy and increased

detection of epithelial abnormalities (Austin RM et al 1998)[46] (Baker et al 2002)[47].

Two types of LBC are in use. The First generation LBC & Second generation LBC.

A. FIRST GENERATION LIQUID BASED CYTOLOGY

Liquid-based cytology (LBC) was introduced in the mid-1990s. A number of different LBC techniques are in use worldwide. These include ThinPrep, SurePath, Cytoscreen, Cyteasy, Labonord Easy Prep, Cytoslide, SpinThin, AutoCyte and PapSpin. Of these the first two methods are approved by FDA and are widely used worldwide. Both have also been used for nongynecological cytology (Yukihiro Kobayashi et al 2011)[48].

i. Thin Prep Method

In ThinPrep method specimens are collected by using Cervex-Brushes. Each brush is rinsed in a vial of PreservCyt solution which is methanol based fixative and preservative fluid, by pressing it into the bottom of the vial .The preservative liquid probably consist of buffered cell mediums with a relatively low alcohol content as it must fulfill both the requirements of a cell transport medium and a cell fixative.

Further processing of specimen is carried out in the ThinPrep automated processor in which the clumps of cells and mucus are broken up by mechanical agitation. Then the liquid preservative solution is

filtered through a membrane filter with a pore size specifically designed to trap epithelial cells while allowing contaminating red blood cells and inflammatory cells to pass through. The epithelial cells collected on the membrane filter are then transferred onto a glass slide in circle of 20mm diameter. After that the slides are dried and stained by automated stainer. This produces a relatively thin, monolayer-type preparation (Abulafia O et al 2003)[49] (Park et al 2001)[50].

Numerous studies have been done to evaluate the efficacy of this ThinPrep method. A study by Bernstein Sara J et al(2001)[51] showed that the ThinPrep test provide more number of adequate smears & detect more cases of squamous intraepithelial lesions than CS. There is no difference in the rate detection of ASCUS between the two methods.

Another comparative study by Annie N. Y. Cheung et al (2003)[52] showed that TP method increases the number of satisfactory smear compared to CS .It also increases the rate of detection of intraepithelial lesions. This shows that TP method is highly effective method of cervical cancer screening.

ii. Sure Path Method

This system works on the principle of density gradient. In this method samples are collected with a broomlike device with a detachable head .Head of the brush is removed from its stem and placed into a vial of ethanol based fixative. In SurePath method clumps of cells and mucus

are broken up by aspiration through a syringe. The cell suspension is then layered on top of a density gradient and the red blood cells and inflammatory cells are separated from the epithelial cells by density gradient centrifugation.Then the cell pellet is resuspended and transferred to a glass microscope slide in 13 mm circlular area(Colgan TJ et al 2004)[53].

Numerous studies compared the diagnostic performance of SurePath LBC technique with CS.A study by B.Kirschner et al (2006)[54] showed that SurePath method reduces the rate of unsatisfactory smears . But smears without endocervical component were increased.The percentage of samples with atypical cells and cells suspicious for malignancy were also increased. This study showed that TP method detects more number of precancerous lesions.

Another comparative study by Maurice Fremont-Smith et al (2004)[55] showed that SurePath method detect more cases of intraepithelial lesions than CS and provides more number of adequate smears.

Staining

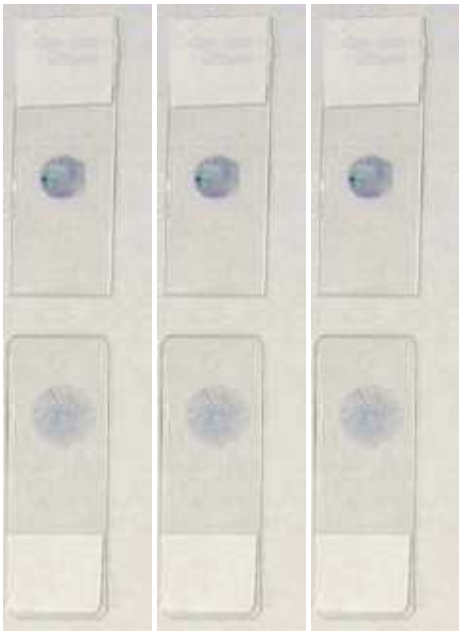
The two system of LBC uses slightly different approach to staining. With the thin prep method slides are stained by using automated staining machine and protocols for staining is similar to that of conventional smears. In SurePath method, staining is an integral part of

process and results are slightly different from the conventional cytology with regards to cytoplasmic staining.

The following table depicts the difference between ThinPrep &SurePath method**(TABLE 2)**

TABLE 2: DIFFERENCE BETWEEN THINPREP AND SUREPATH METHOD

	THINPREP	**SUREPATH**
Collecting Device	Brush is washed in the fixative and discarded	Bristle is detached into the fixative
Name of Fixative	PreserveCyt fluid	CytoRich fluid
Fixative Component	Methanol	Ethanol
Vortex	No vortex	Vortex mixed
Gradient Centrifuge	No gradient centrifugation	gradient centrifugation
Sedimentation	No sedimentation	Sedimentation
Filter	Filter used	No Filter used
Staining	Standard automated staining	Integral part of procedure
Smear Area	20mm	13mm



SUREPATH SLIDES-13mm

THIN PREP SLIDES-20mm

FIG 4: COMPARISION OF SUREPATH & THINPREP SLIDES

Few studies have compared the 2 LBC techniques in terms of accuracy, rate of satisfactory cytology and sufficiency of residual material for HPV DNA testing .A study conducted by Fang-Hui Zhao, et al (2011)[14] showed that both methods yield similar rate of detection of cervical cancer. However, SurePath method provides greater reduction in the rates of unsatisfactory smears and provides sufficient residual material for HPV testing. This is because SurePath cell enrichment process was able to handle significantly greater amount of mucus & blood than ThinPrep membrane filtration process.

Advantages of LBC

Advantages of LBC over conventional cervical cytology are

1. More representative transfer of cells from the collection device to the slide.
2. Reduces the rate of unsatisfactory cytology smears.
3. The availability of residual cellular material for ancillary studies.
4. Reduces the inflammatory cell background. Hence epithelial cell morphology can be better evaluated.
5. A possible reduction in specimen interpretation time.

Disadvantages

The first generation LBC systems (ie:-ThinPrep and SurePath) requires

1. Automated equipment, plastic devices, filters and vacuums.
2. High cost per slide.
3. Cytological interpretation differs from conventional methods and users have got to be trained.

   To address these limitations of the first generation Liquid Based Cytology, a Second generation LBC was introduced.

B.SECOND GENERATION LIQUID BASED CYTOLOGY

The second generation of liquid-based cytology system named LiquiPrep ,eliminates most of the instruments required by the first generation tests thereby offer a simpler method with lower costs for cervical cancer screening(Jongkolnee Settakorn J et al 2008) [16].

LIQUI PREP (LP) SYSTEM

This has been introduced recently & it is designed to match the features and benefits of first generation LBC but address the major issues of instrumentation and cost.

LP system consist of

1. Specimen preservative
2. Specimen cleaner
3. Cell base reagent.

Procedure For Specimen Collection And Processing

Excess cervical mucus is removed using cotton swab and the cervical brush is inserted into the cervical canal & rotated 3- 5times in clockwise direction .The brush head is detached into the vial containing 5ml of preservative fluid which is an alcohol based fixative. The specimen containing cervical brush and preservative is mixed with the vortex to form a homogenous mixture. Then 4 ml of cleaning solution is taken in to a tube and entire content of the fixative vial is poured in to the tube and centrifuged at 1000 g for 10 min. This cleaning solution separate

the cells from the mucus and blood .The supernatant is discarded .To the cell pellet, the cell base is added (4-5 volumes of cell button) and fully suspended by vortex mixing .50 µl of mixture is pippetted onto a slide in a circular motion (15-17mm) and the slides are dried at room temperature and stained with Pap stain (Jongkolnee Settakorn et al 2008)[16]Hao Deshou et al (2009) .[56]

There are various studies showing the efficacy of LP system. The study by Roghaei MA et al (2010)[57] showed that LP method increases the number of satisfactory smears (62.4%) compared to CS (31.9%). This study also states that, LiquidPrep is an inexpensive method, relying on cell handling procedures. The number of cells transferred to the slide is controlled by the cytologist.A study by Hao Deshou et al (2009) [56]showed that LP method detect more cases of intraepithelial lesions of cervix compared to CS.

A comparative study conducted by Mahmood Khaniki et al (2009) [58]showed that LiquiPrep samples (94.7%) were more adequate than CS (92.1%).The LiquiPrep method provided significantly higher sensitivity (83% vs. 66%) than the CS to detect SIL at histology but the difference in specificity was not significant (98% vs. 86%).

Another study conducted by M Tunc Canda et al (2010) [59]showed that LP method reduces the number of unsatisfactory smears and increases the detection rate for atypical squamous cells. The rate of

detection of LSIL & HSIL was also increased with LP. The LP method detected more squamous cell lesions than CS.

Alves et al (2004)[60]compared the different types of LBC techniques based on the morphological details like cellular adequacy, clean background, cell overlapping ,uniform distribution ,cytoplasmic &nuclear changes and presence of inflammatory cells etc. This study highlights that all these methods provide adequately preserved cellular structure & choice of the method depends on cost & availability of the procedure.

Advantages of LP Method

The LP method provides a clean background with better preservation of cells compared to CS. The area for examination of slide was also reduced thereby decreases the screening time. Cost comparison of LP is higher than the CS but less expensive than 1st generation LBC method. Ancillary studies particularly for HPV can be done on the same residual samples. This feature makes LiquiPrep system apt for cervical cytology screening in developing countries (Park et al 2007)[61] (Jongkolnee Settakorn J et al 2008) [16](M Tunc Canda et al 2010) [59].

3. MANUAL LIQUID BASED CYTOLOGY

One of the major limitations of LP method compared to CS is its higher cost. To overcome this, few studies have been done on manual membrane liquid based cytology technique. In this method cervical

cytology smears are processed by using a fixative and cell encapsulating polymer solution prepared in their own laboratory .The smears are prepared with the use of simple equipment –a vortex and laboratory centrifuge (Maksem et al 2001) [17](Maksem et al 2005) [18] (Lee et al 2006) [19](Kavatkar et al 2008) [20] (Nandini et al 2012) [21].

In 2001 Maksem et al reported on the formulation of an alcohol-agar solution for manual slide preparation. An aqueous blend of nutrient agar, linear alcoholic alkoxylate - a surface wetting agent, polyethylene glycol and reagent alcohol are mixed together in an appropriate ratio to form a viscous solution that mixes with all types of cytology fixative to form a uniform viscous suspension. On spreading across a glass slide, this produces a monolayer sheet of cells. In his study cytology specimens were fixed with commercially available fixative which is then transferred into alcohol-agar in a test tube. The tubes were centrifuged at 600g for 10 minute and supernatant was discarded. Vortex mixing of cell pellet produces a gel to sol transition to form a cell suspension, from which smears are prepared. The slides showed un clumped monolayer sheets of cells with good preservation of cellular morphology. He found that only 0.2% of smears are unsatisfactory which was solely attributed to inadequate sampling. He also noted that there was 3 fold increase in the detection of SIL & 45% reduction of ASCUS diagnosis compared to previous year statistics.

This inexpensive method is based on Saccomano's technique for sputum processing. The difference of the MLBC from Saccomano's techniques involves substitution of vortex mixer for a mechanical blender and addition of nutrient agar, glycerin and linear alcohol alkoxylate to a PEG-alcohol solution (Maksem JA et al 2001) [17].

In 2005 Maksem et al again reported a technical improvement in MLBC method. An improved polymer-Gel solution was prepared by using DNA –grade agarose, PEG and 0.1% poly-L-lysine solution which can be stable for 2 years[18].

In his study he also found that most of the discrepancies between Automated LBC & MLBC method may be related to

1. The size of the screened area
2. Number of slides examined
3. MLBC'S capacity to retain microbiopsies on glass slide.

In 2006 Lee et al conducted a split sample study to validate MLBC method for cervical smear preparation. In his study, the cells suspended in polymer solution were spreaded over the slide to cover a circular area of 20-25 mm in diameter. Later the slides are air dried and stained with Papanicolaou stain. He noted that there was 76.3% overall agreement between MLBC & CS. In addition MLBC method was highly sensitive method of detecting cervical lesions compared to CS[19].

Anita N Kavatkar et al (2008)[20] their study prepared cervical cytology smears using the manual method. The samples were fixed in a fixative prepared in their own laboratory by using alcohol, water, sodium chloride &10% formalin. After that specimen was vortex mixed and centrifuged at 800g for 10 min. The supernatant was discarded and 2 ml of alcohol-agar solution containing agarose, polyethylene glycol, alcohol, and poly-L-Lysin was added. Again the specimen was vortex mixed and 3-6 drops of suspension was placed on a glass slide and allowed to spread. On drying, polymer solution forms monolayer sheet of cells which are sealed in to partly soluble membrane that hold the cells on to the glass slide. They found that MLBC method was comparable to conventional smears.

NM Nandini et al (2012)[21] compared MLBC method with CS & histopathology. They adopted the same method of Kavatkar et al for preparation of fixative ,cell base & compared the morphological features of the both preparation .They found that MLBC method detected more precursor lesions by providing good morphological details, compared to CS.

The best prevention programs should be determined regionally on the basis of local resources and acceptability. Hence this low cost MLBC can be used as screening method in resource limited settings (Schiffman and Castle 2005) [62].

ASSESSMENT OF LIQUID-BASED CYTOLOGY SMEARS

The screening fields of the slide are round-shaped and much smaller than the fields in conventional preparations. Screening methods therefore differ from those used for conventional smear preparations

A. Adequacy Criteria For Liquid-Based Cytology Preparations

The minimum requirement in liquid-based samples is the presence of 5000 well-preserved and well visualized squamous cells. In contrast, cellular adequacy of conventional Pap smears is based on the assessment of the cellular pattern on the slide; cell counts are not recommended in CS. For LBC a minimum of 10 microscopic fields should be assessed (Solomon D 2004)[63].

The number of cells required per field = 5000/(area of preparation/area of field. For both conventional smears and LBC an adequate transformation zone component requires at least ten well preserved endocervical cells. Satisfactory specimen reports should include a comment on the presence or absence of endocervical component (George G. Birdsong et al 2004) [64.]

B. Cell Morphology of Liquid-Based Cytological Preparations In Comparison To Conventional Preparations

Liquid-based cytology preparations are very similar to those of the regular smear method, although their morphology can differ slightly.

1. The most important difference is the clear background of liquid cytology preparations which enables an easy visual access to abnormal cells to facilitate their interpretation.
2. There is less blood, fibrin and necrotic debris on the slides due to their special processing.
3. Tumor Diathesis consisting of blood, fine granules of fibrin and necrotic debris is found in a discrete pattern .Diathesis hang like wallpaper on the surface of the cells and cell structures ("clinging diathesis").
4. Generally LBC preparations have less nuclear enlargement than conventional smears due to immediate fixation .Naked nuclei from autolysis may be reduced in number.
5. Squamous metaplastic cells in LBC preparations shows increased N/C ratio due to rounding up of cells which may mimic HSIL. Features favoring metaplasia are an increased N:C ratio less than 50% of cell, smooth nuclear contour and even distribution of chromatin(Sherman ME et al 2001) [65].
6. In LBC preparations endometrial cells appear slightly larger with more obvious nucleoli and enhanced chromatin details than conventional smears. They appear above the plane of squamous epithelial cells, either as groups or single cells with prominent intra

cytoplasmic vacuoles and bean shaped nuclei in a cleaner background.

7. Atypical squamous cells denotes cytological changes favour SIL which are qualitatively and quantitatively insufficient for a definitive interpretation(Solomon D et al 2002)[66].The interpretation of ASC requires 3 Features
    a. Squamous differentiation
    b. Increased N:C ratio with 2-3 times the size of nucleus of intermediate cells
    c. Minimal nuclear hyperchromasia, irregularity & multinucleation.

    The appearance of ASC-US in CS and LBC is similar .In CS the cells may appear large and flatter. In LBC smears, ASC-H cells may appear small with nuclei 2-3 times the size of neutrophil nucleus.
8. LSIL typically involves mature squamous cells with intermediate or superficial type. Cells of HSIL have more immature type cytoplasm .Overall cell size is smaller in HSIL as compared with LSIL.
9. The squamous cell carcinoma should be recognized due to its characteristic morphology and not due to tumour diathesis. LBC preparations are often characterized by lower tumour cellularity

(Clark SB et al 2002) [67].Tumour diathesis and invasive features may be difficult to discern in LBC smears resulting in some cancers being interpreted as HSIL (Renshaw AA et al 2004)[68].

TABLE 3: FEATURES OF KERATINIZING AND NON KERATINIZING SCC IN LBC PREPARATION

CORNIFIED TYPE(IN LBC)	NONCORNIFIED TYPE(IN LBC)
There is no difference in keratinized cells in comparison to conventional preparations	• Cytoplasm is more contracted &denser • The nucleus appears smaller • Chromatin is distributed more evenly • Nucleoli are more prominent • Malignant nuclear features are preserved

C. Cytomorphology of HPV Infection Using Liquid-Based Cytology

Both classical HPV sign (koilocytes) and non classic signs such as abortive koilocytosis, mild dyskeratosis, parakeratosis, mild nuclear hyperchromasia, pointed nuclei, grooved nuclei, multinuclear cells, keratohyalin-like granule cells, and condensed cytoplasmic filaments are better appreciated in LBC preparations. These secondary HPV signs have

a negative predictive value of 100 %. If they are missing, it is highly probable that the woman is HPV negative (Bollmann et al. 2005) [69].

HPV DNA TESTING

HPV plays a central role in the development of carcinoma of cervix.HPV DNA testing can be done on the residual material obtained from LBC preparations.

Various methods of HPV detection are

1. Simple scoring of koilocytes
2. IHC staining
3. Dot –blot
4. Southern blot
5. In –situ-hybridization
6. The Hybrid Capture Assay
7. PCR

Hybrid Capture assay

This system works on the principle of nucleic acid hybridization assay with signal amplification for the qualitative detection of DNA of high-risk type. Since it is based on signal rather than amplification, it is less prone to cross-contamination.

CYTOLOGICAL TERMINOLOGY

The terminology used in cervicovaginal cytology has evolved over the course of years. The first system introduced in 1954 was the Papanicolaou classes.

A.PAPANICOLAOU CLASSES

This system consist of five classes

Class	Description
I	Absence of atypical or abnormal cells
II	Atypical cytology, but no evidence for malignancy
III	Cytology suggestive of, but not conclusive for, malignancy
IV	Cytology strongly suggestive of malignancy
V	Cytology conclusive for malignancy

From Papanicolaou, 1954

Although this nomenclature fulfilled a very important role in the establishment of the technique resulting in standardized format of reporting, it was eventually abandoned because of the vagueness of the information provided. It had also lacks equivalent terminologies for histopathologically diagnosed lesions and does not mention about non Neoplastic condition (Seybolt JF et al 1971)[70].Then WHO terminology was introduced.

B.WORLD HEALTH ORGANIZATION TERMINOLOGY

The WHO terminology allows more precise correlation between cytological and histopathological findings (Riotten et al 1973)[71]. It includes a number of different entities. These are

1. Mild dysplasia
2. Moderate dysplasia
3. Severe dysplasia
4. Epidermoid carcinoma in situ
5. Epidermoid carcinoma in situ with minimal stromal invasion
6. Invasive epidermoid microcarcinoma
7. Invasive epidermoid carcinoma.

But there are many disadvantages in WHO terminologies. Studies have shown high rates of intra-observer and inter-observer variation with cervical cytology .Other limitations of the WHO terminology are that it does not adequately deal with non neoplastic conditions nor with specimen adequacy (Sherman ME et al 2001)[65].

As a result of better understanding of the pathogenesis of cervical cancer, the cervical intraepithelial neoplasia (CIN) terminology was introduced in the late 1960s (Richard 1973)[31] .

C. CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN)TERMINOLOGY

The CIN concept emphasizes that dysplasia and carcinoma in situ represents different stages of the same biological process. It had a major impact on how precancerous lesions are treated, since all types of cervical cancer precursor are considered to form a biological and clinical continuum.

The CIN terminology includes

1. CIN 1
2. CIN 2
3. CIN 3

The CIN terminology is still widely used in many countries for reporting both histological and cytological diagnoses.

D. THE BETHESDA SYSTEM TERMINOLOGY

This was introduced in 1988, by the US National Institutes of Health conference in Bethesda, Maryland to develop a new terminology to provide better standardization and uniform reporting of Pap smears. This terminology is known as The Bethesda System (TBS). On the basis of experience obtained during the first three years of its use in 1991 the Bethesda System was slightly modified. After the invent of role of HPV in the pathogenesis of cervical neoplasia TBS was once again revised. Additionally, algorithms for the treatment and follow-up of intraepithelial

lesions were inconsistent. As a result, in April 2001 the third Bethesda conference convened, to update the 10- year-old system and it was further modified and The Bethesda System 2001 was developed. **(Appendix 1)** (Solomon et al 2002) [66].

The overall structure of the TBS 2001 reporting system is similar to the previous system (TBS 1991) but there are several important changes. That includes

1. The report is considered to be an ‘‘interpretation’’ and not a diagnosis.
2. The adequacy statement of ‘‘satisfactory but limited by’’ has been dropped. The Pap test is now interpreted either satisfactory or unsatisfactory for evaluation and not further classified according to a limitation.
3. All negative Pap tests are reported under the general interpretation of ‘‘negative for intraepithelial lesion or malignancy,’’ or ‘‘NILM.’’ This term may be compared with the finding of organisms, reactive changes, and other benign findings, in contrast to the previous system, whereby ‘‘within normal limits’’ /Benign cellular changes was reported alone.
4. The categories of ‘Infection’ are changed to ‘Organism’.
5. The reporting of benign reactive changes is optional. Documentation of reactive changes in the report to spot trends in a

series of cervical cytology specimen from same patient. Some studies showed a mild increase in the incidence of SIL in cases interpreted as reactive compared to that reported as within normal limits. This helps in future studies (Mali SN et al 2001) [72].

TABLE:4 COMPARISION OF VARIOUS CYTOLOGICAL TERMINOLOGIES

PAPANICOLAOU CLASSES	**WHO**	**CIN**	**THE BETHESDA SYSTEM**
Class 1			Within normal limits
Class 2			BCC ,ASC
Class 3	Mild dysplasia Moderate dysplasia Severe dysplasia	CIN I CIN II CIN III	LSIL HSIL
Class 4	Carcinoma in situ	CIN II	
Class 5	Microinvasive carcinoma	Invasive carcinoma	Invasive carcinoma

MATERIALS AND METHODS

This study was conducted in the Department of Pathology at Tirunelveli Medical College from August 2010 to April 2012.A split sample study was done and approval of the Ethical committee of Tirunelveli Medical College & Hospital was obtained.

In our study we proposed to conduct a comparative analysis of cervical cytology by using (a) conventional Pap smear with manual liquid based cytology and (b) conventional smears with LiquiPrep method. Samples were collected from the patients attending the Gynaecology Outpatient Department after obtaining consent. The patients presenting with white discharge, post menopausal bleeding, unhealthy cervix on speculum examination were included in our study(**Appendix 2**).Totally 150 samples were studied .100 cases were analyzed by MLBC .Of these, 50 case were subjected to different concentration of fixative & cellular base for standardization. The remaining 50 cases were subjected to comparative analysis of manual liquid based cytology with Conventional pap smear. The other 50 cases were analyzed &compared by LiquiPrep and Conventional method.

I. LIQUI PREP METHOD

A cervical brush provided by the manufacturer was used for collecting specimens for LiquiPrep preparations .The brush was inserted in the endocervical canal while the patient was in lithotomy position & rotated to 360 degree 2-4 times .After that conventional smear was prepared by touching the brush on to the slide. Then the bristle was detached from the stem and put into the vial containing 5ml of alcohol based fixative fluid and the sample was send to the laboratory for further processing.

In the laboratory the sample was mixed with vortex till it becomes a homogenous mixture which will take 5 -10 mins. Then 3ml of cleaning solution was added to the specimen. This will remove the mucus and blood from the specimen that obscures the cellular morphology. This mixture was centrifuged at 800 rpm for 10 minutes. The supernatant was discarded. To the cell pellet at the base of the tube, 1.5 ml of cellular base was added. This mixture was once again mixed with vortex. With the help of the micropipette 50 µl of the suspensions was taken and placed over the slide in a circular manner. The slides are air dried and stained with Rapid pap stain.

II. MANUAL LIQUID BASED CYTOLOGY

For MLBC fixative and cell base were prepared in our laboratory.

Fixative

Fixative was prepared by using absolute alcohol, 10% formalin, sodium citrate and sodium chloride.

Cell Base (Alcohol-Agar Polymer Suspension)

The main purpose of Cell base is to suspend the cells in a monolayer sheets. This was prepared by using Agarose, Poly ethylene glycol, Absolute alcohol and Poly-L-Lysine.

1. 1 gram of Agarose was added to 75 ml of deiodinized water in a beaker and mixed to an even suspension .The suspension is then boiled, until a yellow coloured clear suspension is obtained.
2. Poly ethylene glycol(PEG)
3. Poly -L-Lysine –10 mg of L-lysine is dissolved in 100ml of distilled water to obtain 0.1% solution.
4. Absolute alcohol

STANDARDIZATION OF FIXATIVE & CELL BASE FOR MLBC

STANDARDIZATION METHOD I

Initially we prepared a fixative and cell base in the following ratio.20 cases were evaluated by using this fixative & cell base.

TABLE: 5 COMPOSITION OF FIXATIVE -METHOD I

COMPOSITION OF FIXATIVE	RATIO
10% formalin Sodium citrate Sodium chloride Absolute alcohol	1 (5ml):1 (5ml):1 (5ml): 17 (85 ml)

TABLE: 6 COMPOSITION OF CELL BASE-METHOD I

COMPOSITION OF CELL BASE	RATIO
Agarose PEG L-lysine	1 (30ml):1(30ml):0.5 (15ml)

Final volume of 100 ml is obtained by diluting it with 25 ml of absolute alcohol

STANDARDIZATION METHOD II

Later on glacial acetic acid was added to the fixative in an attempt to remove the RBC's & inflammatory cells in the background. We adjusted the ratio of agarose and PEG in the cell base to hasten the process of drying of smears. 20 cases were studied by using this fixative & cell base.

TABLE:7 COMPOSITION OF FIXATIVE –METHOD II

COMPOSITION OF FIXATIVE	RATIO
10% formalin Sodium citrate Sodium chloride Glacial acetic acid Absolute alcohol	1(5ml):1(5ml):0.5(2.5ml):0.5(2.5ml):17(85ml)

TABLE :8 COMPOSITION OF CELL BASE-METHOD II

COMPOSITION OF CELL BASE	RATIO
Agarose L-lysine PEG	1 (15ml): 1 (15ml):2 (30ml)

STANDARDIZATION METHOD III

Then the concentration of alcohol was gradually increased in an attempt to remove/ dissolve mucus, based on Saccomano's method of sputum processing. Concentration of agarose in the cell base was increased to further decrease the drying time of the smears .10 cases were subjected for standardizing this fixative & cell base.

TABLE: 9 COMPOSITION OF FIXATIVE -METHOD III

COMPOSITION OF FIXATIVE	RATIO
10% formalin Glacial acetic acid Absolute alcohol	1 (2.5ml):1 (2.5ml): 19 (95ml)

.

TABLE: 10 COMPOSITION OF CELL BASE-METHOD III

COMPOSITION OF CELL BASE	RATIO
Agarose PEG	3 (45ml):2(35ml)

The 50 cases of Manual LBC were analyzed in this study by method III.

PROCEDURE FOR PREPARATION OF SLIDES

1. Samples are collected by using wooden spatula .The spatula was inserted into cervical canal and rotated to 360 degrees. The head of the spatula was broken into a vial containing 4 ml of fixative and fixed for 1-4 hours.
2. The fixative solution with cervical scrape sample was mixed thoroughly to obtain a homogenous mixture.
3. This mixture was then centrifuged at 800 rpm for 10 min
4. The supernatant was discarded and 1-2 ml of polymer solution was added.
5. This was further mixed thoroughly to obtain a homogenous suspension.
6. 2 drops of suspension was pippetted and placed over a glass slide. With the help of another slide the drops were spread out in a homogenous layer .Then the slides are pulled apart, so that the cells will be equally represented on both slides.
7. The slides were then air dried and stained with Rapid Pap stain.

RAPID PAP STAINING

1. Dip the slides in nuclear stain- hematoxylin -2 min
2. Wash in Scott's tap water buffer for 30 seconds.
3. Dip in rapid pap dehydrant I & II each for 30 seconds
4. Dip in Working cytoplasmic stain for 1 ½ min

5. Repeat dehydration for 30 seconds
6. Air dry the smears
7. Dip in xylene and mount in DPX

INTERPRETATION

Nucleus-blue

Keratinized cells-pink/orange

Squamous cells prior to keratinisation-sky blue/light green

RBC-salmon pink

WBC- blue

Mucus-blue/pink

MORPHOLOGICAL PARAMETERS ASSESSED

The morphological parameters studied includes

1. Cellularity(adequate/inadequate)
2. Clean background(present/absent)
3. Uniform distribution(present/absent)
4. Cellular overlapping(present/absent)
5. Inflammatory cell background(present/absent)
6. Cytoplasmic distortion(present/absent)
7. Nuclear irregularity(present/absent
8. Final interpretation(Based on The Bethesda System 2001)

The LiquiPrep slides were assessed on above criteria. MLBC smears were also assessed with same criteria except for cellularity which was graded in to 3 grades (1,2,3) based on number of cells in each 40X field.

GRADE 1- up to 150 cells- Inadequate for reporting

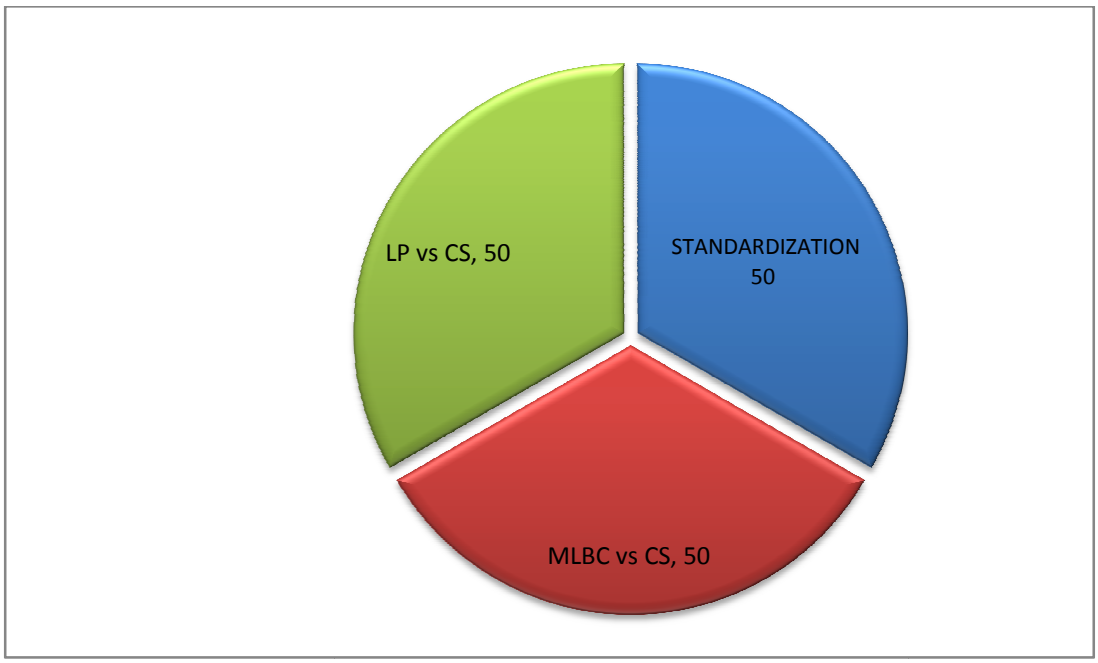
GRADE 2-150- 500 cells-Just adequate

GRADE 3- ≥ 500 cells-Adequate

OBSERVATION AND RESULTS

This study was conducted in the Department of Pathology at Tirunelveli Medical College from August 2010 to April 2012. 150 cases of cervical smears were collected. 50 of these cases were used for standardization of MLBC technique and excluded from the study. 50 cases were analyzed by MLBC with comparative conventional smears for various morphological features and final interpretation. The remaining 50 cases were subjected to LiquiPrep cytology along with conventional smear study **(CHART1).**

CHART: 1 DISTRIBUTION OF CASES



The smears were studied by using 7 morphological parameters. For reporting ,The Bethesda system2001 was used in both methods.

I. MANUAL LBC versus CONVENTIONAL SMEARS

For Manual LBC we had to standardize fixative and cell base solution. We required 50 cases for standardization and the following observations are made.

METHOD I OF STANDARDIZATION OF SOLUTION

In the first method we used the fixative & cell base in the ratio depicted in **TABLE 5 &6**. The observations are

1. Cells shows shrinkage artifact.
2. Nuclear & cytoplasmic details are distorted and not clearly made out.
3. Background shows abundant mucus & blood which obscures the cellular details.
4. Smears do not dry quickly.
5. Smears easily washed off while staining.

METHOD II OF STANDARDIZATION

An attempt was made to remove the inflammatory background by adding glacial acetic acid to the fixative. The ratio of agarose and PEG in cell base was altered in an attempt to hasten the process of drying of smears (**TABLE 7&8).**The observations are

1. Inflammatory cells in the background are reduced
2. Cell shrinkage artifact still present
3. Wisps of mucus in the background present
4. The smears fail to dry.

METHOD III OF STANDARDIZATION

The concentration of alcohol was increased in an attempt to remove/ dissolve mucus, based on Saccomano's method of sputum processing. The concentration of agarose was increased, to further decrease the drying time of the smears. (**TABLE 9&10).**

The observations are

1. Cell shrinkage is reduced.
2. Removes most of the mucus and all the inflammatory cells.
3. Smears dry well with the formation of membrane.
4. Smears does not get washed away.

I.STATISTICAL ANALYSIS OF CS AND MLBC RESULTS

The data regarding Conventional smears vs Manual Liquid based cytology were compared and interpreted by χ^2 (Chi- square) test. The above procedure of statistical analysis and interpretations were made by the statistical software IBM SPSS statistics 20. The P-values <0.05 ($P<0.05$) were treated as significant.

The CS and MLBC smears were compared in respect of cellularity, clean background, uniform distribution, cell overlapping, inflammatory back ground, nuclear distortion, cytoplasmic distortion and interpretation of results.

TABLE-11:COMPARISON OF CELLULARITY

CELLULARITY GRADE	CS	MLBC	TOTAL	x^2	DF	SIGNIFICANCE
1	0	18(36%)	18	49.613	2	P<0.001
2	9(18%)	25(50%)	34			
3	41(82%)	7(14%)	48			
Total	50	50	100			

Of 50 cases, Grade 3 cellularity was seen in only 7 (14%) cases of MLBC slides whereas 41 (82%) cases of CS showed grade 3 cellularity. The difference in cellularity between the procedures was statistically significant (P<0.001) . **(TABLE 11&CHART 2**).

TABLE-12: COMPARISON OF CLEAN BACKGROUND

CLEAN BACK GROUND	CS	MLBC	TOTAL	x^2	DF	SIGNIFICANCE
Present	3(6%)	29(58%)	32	31.066	1	P<0.001
Absent	47(94%)	21(42%)	68			
Total	50	50	100			

In MLBC 29(58%) cases revealed clean back ground compared to 3(6%) cases in CS which was statistically significant (P<0.001).(**TABLE 12 &CHART 3**)

CHART 2

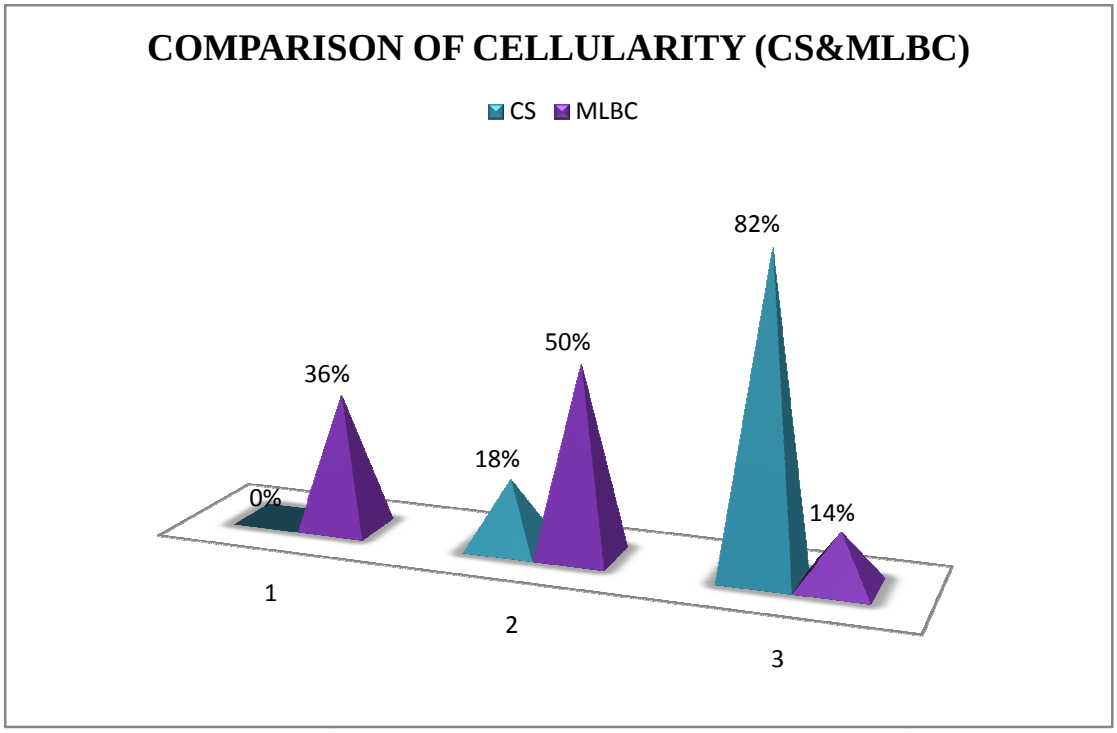


CHART 3

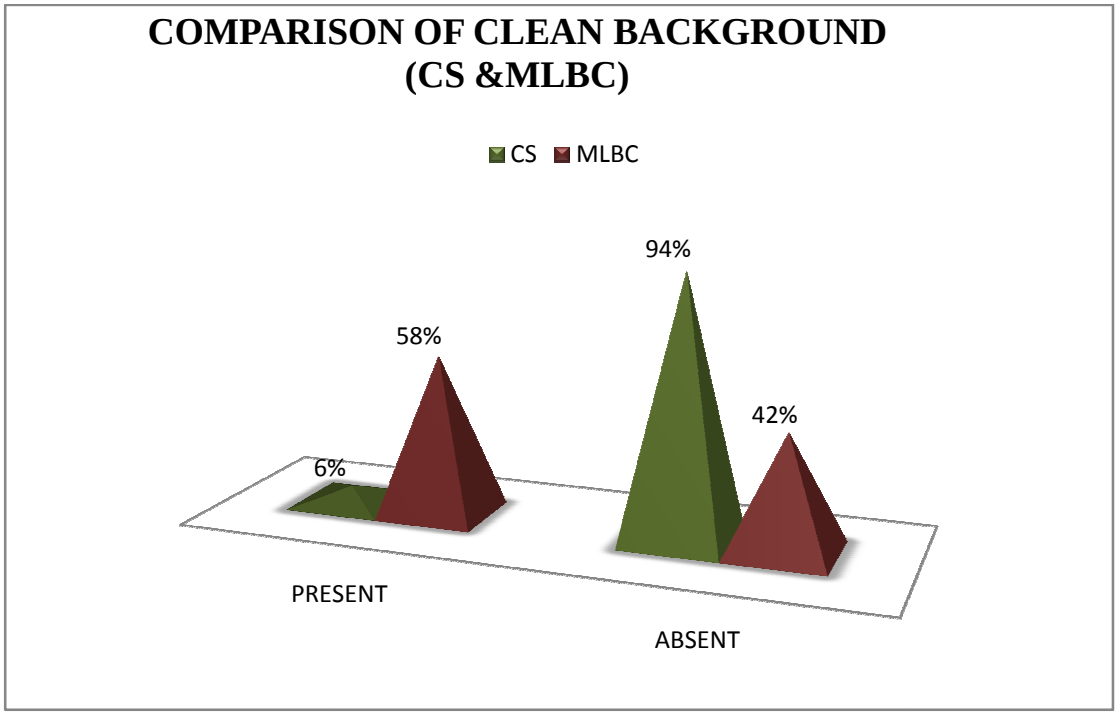


TABLE-13: COMPARISON OF UNIFORM DISTRIBUTION OF CELLS

UNIFORM DISTRIBUTION	CS	MLBC	TOTAL	x^2	DF	SIGNIFICANCE
Present	7(14%)	12(24%)	19	1.624	1	P>0.05
Absent	43(86%)	38(76%)	81			
Total	50	50	100			

12 (24%) cases of MLBC showed uniform distribution where as only 7(14%) cases of CS showed uniform distribution. The difference in uniform distribution between the two procedures was not statistically significant (P>0.05). (**TABLE 13 & CHART 4).**

TABLE-14: COMPARISON OF CELL OVERLAPPING

CELL OVERLAPPING	CS	MLBC	TOTAL	x^2	DF	SIGNIFICANCE
Present	43(86%)	33(66%)	86	5.482	1	P<0.05
Absent	7(14%)	17(34%)	24			
Total	50	50	100			

33 (66%) cases of MLBC smears showed cellular overlapping and 43 (86%) cases of CS showed cellular overlapping. The difference in cell overlapping between two methods was statistically significant P<0.05. CS slides showed more cellular overlapping than MLBC.(**TABLE 14&CHART 5).**

CHART 4

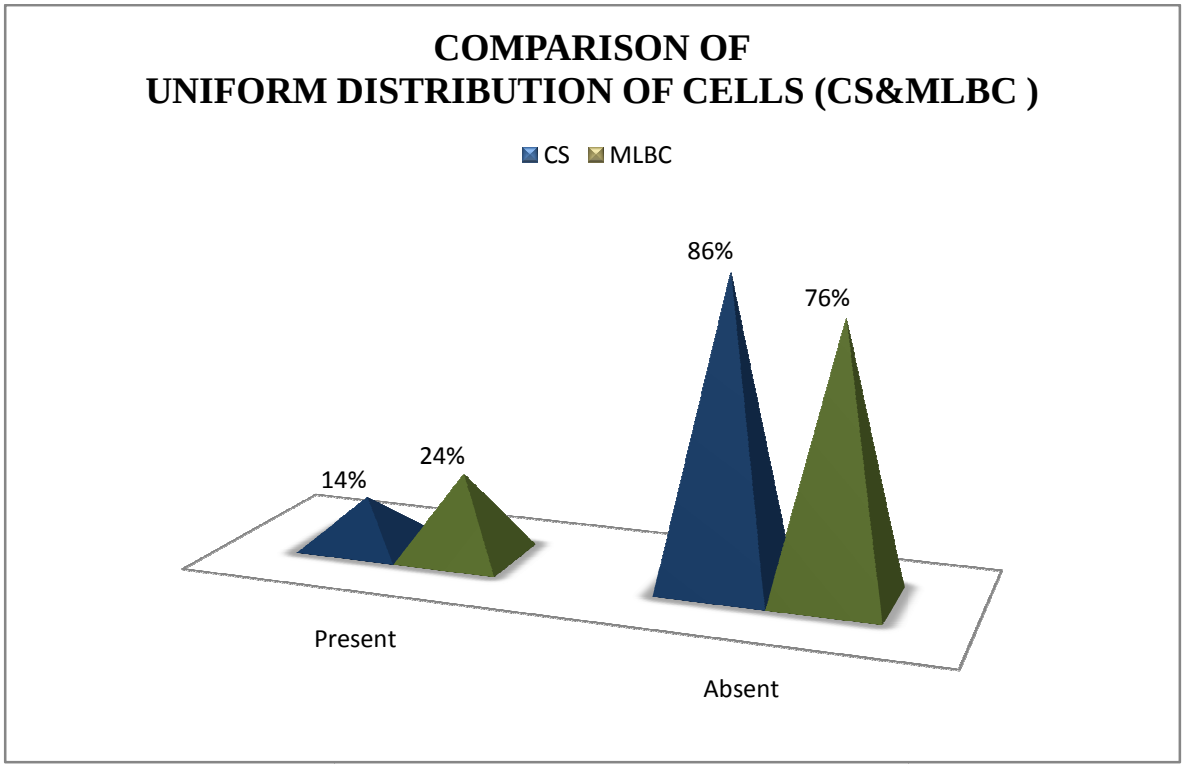


CHART 5

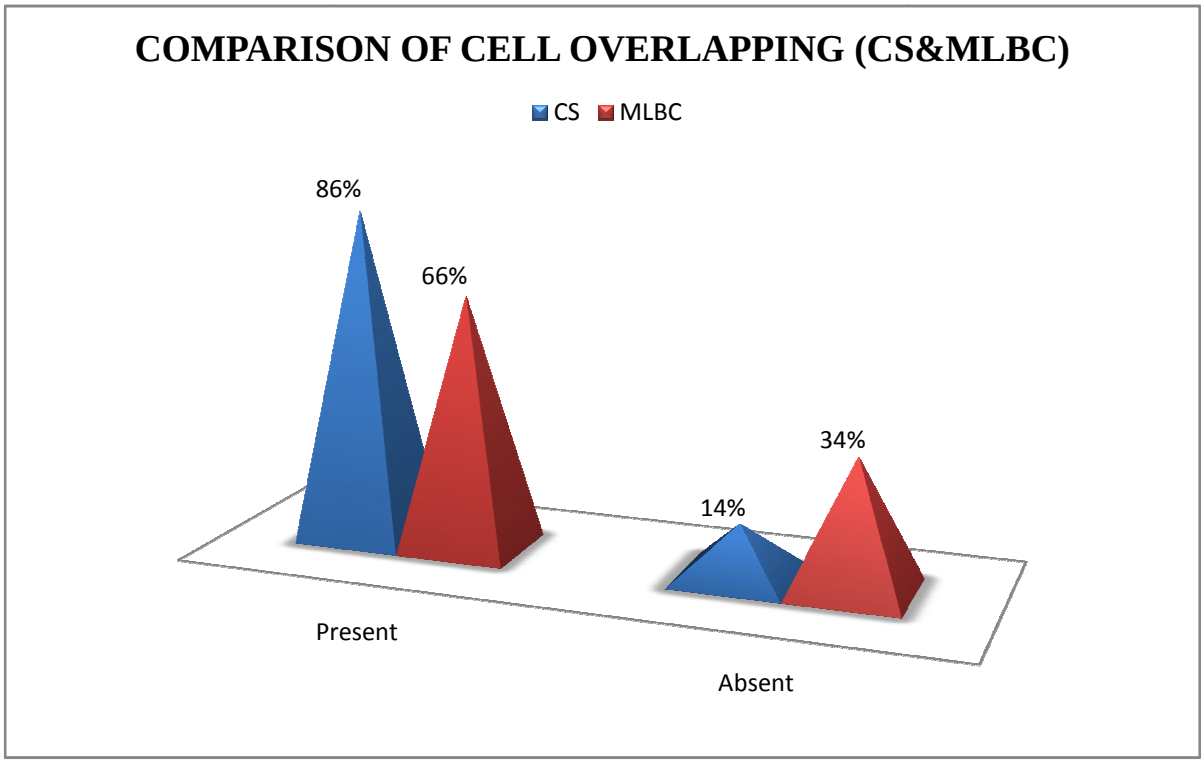


TABLE- 15: COMPARISON OF INFLAMMATORY CELLS IN THE BACKGROUND

INFLAMMATORY BACKGROUND	CS	MLBC	TOTAL	x^2	DF	SIGNIFICANCE
Present	34(68%)	0	34	14.420	1	P<0.001
Absent	16(32%)	50(100%)	66			
Total	50	50	100			

Of the 50 cases none of the MLBC smears showed inflammatory background in contrast to 34 cases (68%)in CS ,which was statistically very significant (P<0.001).(**TABLE 15& CHART 6)**

TABLE- 16. COMPARISON OF CYTOPLASMIC DISTORTION

CYTOPLASMIC DISTORTION	CS	MLBC	TOTAL	x^2	DF	SIGNIFICANCE
Present	6(12%)	24(48%)	30	15.429	1	P<0.001
Absent	44(88%)	26(52%)	70			
Total	50	50	100			

Cytoplasmic distortion was seen in 24 (48%) cases of MLBC preparation in contrast to 6(12%) cases in CS ,which was statistically significant (P<0.001).(**TABLE 16 & CHART 7).**

CHART 6

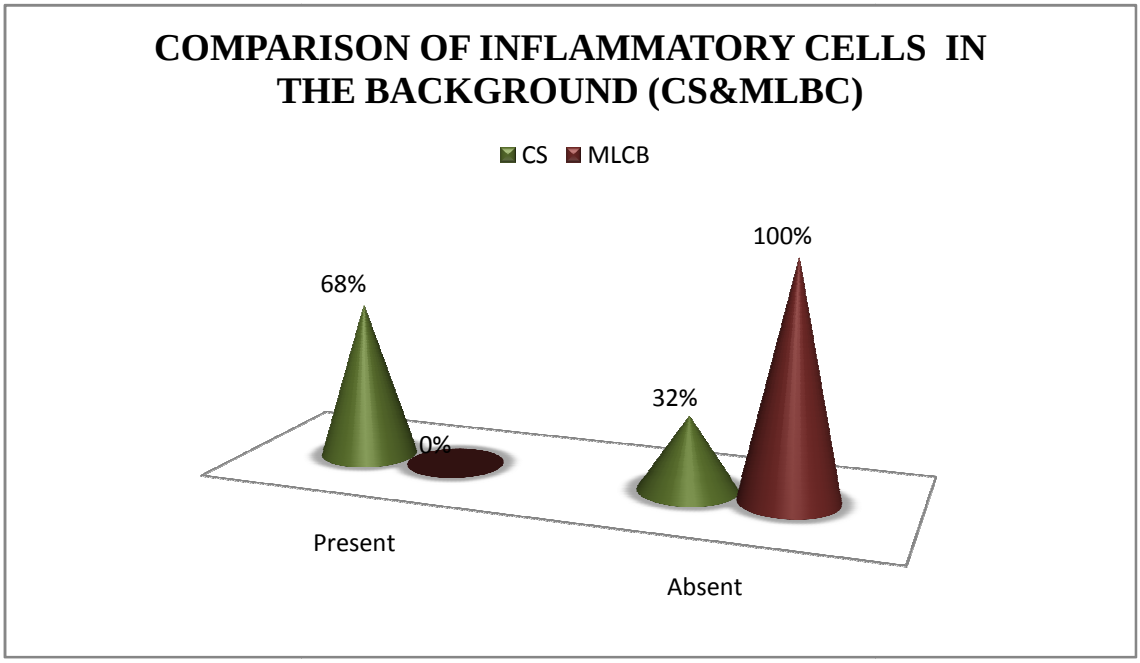


CHART 7

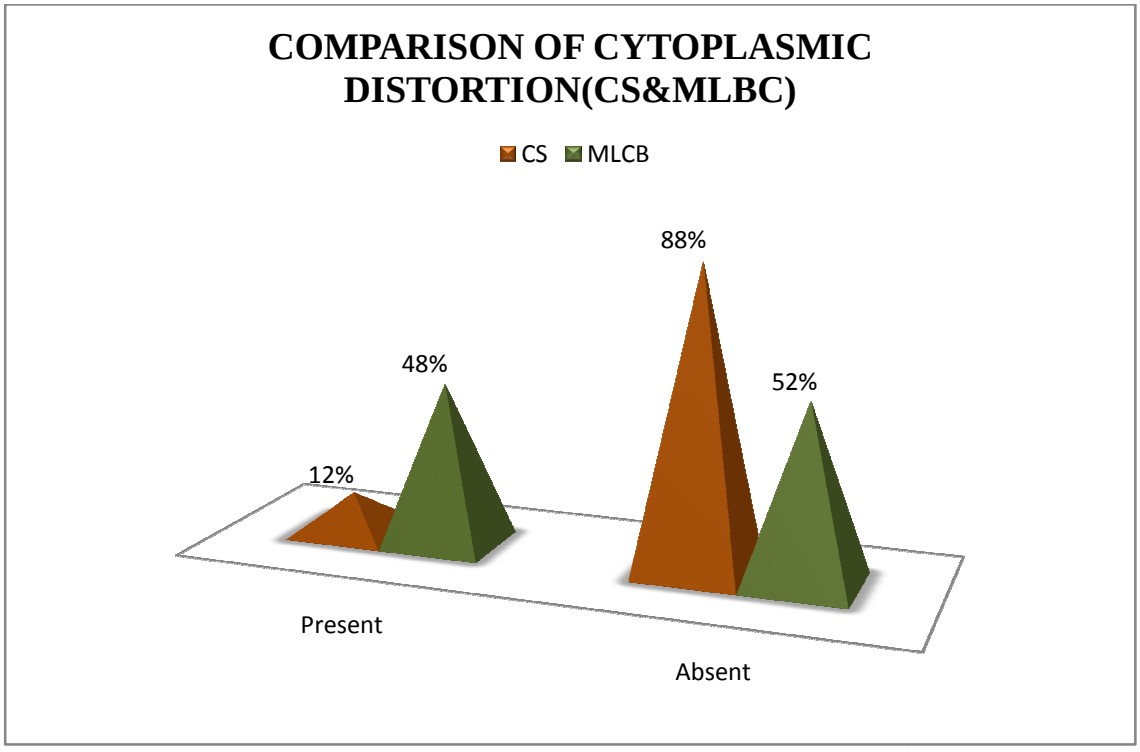


TABLE-17: COMPARISON OF NUCLEAR DISTORTION

NUCLEAR DISTORTION	CS	MLBC	TOTAL	x^2	DF	SIGNIFICANCE
Present	5(10%)	15(30%)	20	6.250	1	P<0.01
Absent	45(90%)	35(70%)	80			
Total	50	50	100			

Of 50 cases, nuclear distortion was present in 15(30%) cases of MBLC smears &5(10%)cases of CS. This was statistically significant (P<0.01) (**TABLE 17 & CHART 8)**

TABLE-18 : INTERPRETATION OF RESULTS BETWEEN THE TWO PROCEDURES.

INTERPRETATION	CS	MLBC	TOTAL	x^2	DF	SIGNIFICANCE
In adequate	0(0%)	18(36%)	18	71.9	5	P<0.001
NILM	9(18%)	32(64%)	41			
NILM-IS	31(62%)	0(0%)	31			
ASCUS	4(8%)	0(0%)	4			
LSIL	4(8%)	0(0%)	4			
HSIL	2(4%)	0(0%)	2			
Total	50	50	100			

In MLBC 18 (36%) cases were reported as inadequate smear but none of the smears were inadequate in CS. The number of inflammatory smears by CS was 31(62%) whereas MLBC does not showed inflammatory cells in any case. In CS 4(8%) cases were reported as ASCUS &LSIL .Two cases were reported as HSIL. By MLBC preparations no intraepithelial lesions were made out. The interpretation of results between the two methods revealed that there was a statistically significant difference between the two methods (P<0.001).**(TABLE 18 &CHART 9).**

CHART 8

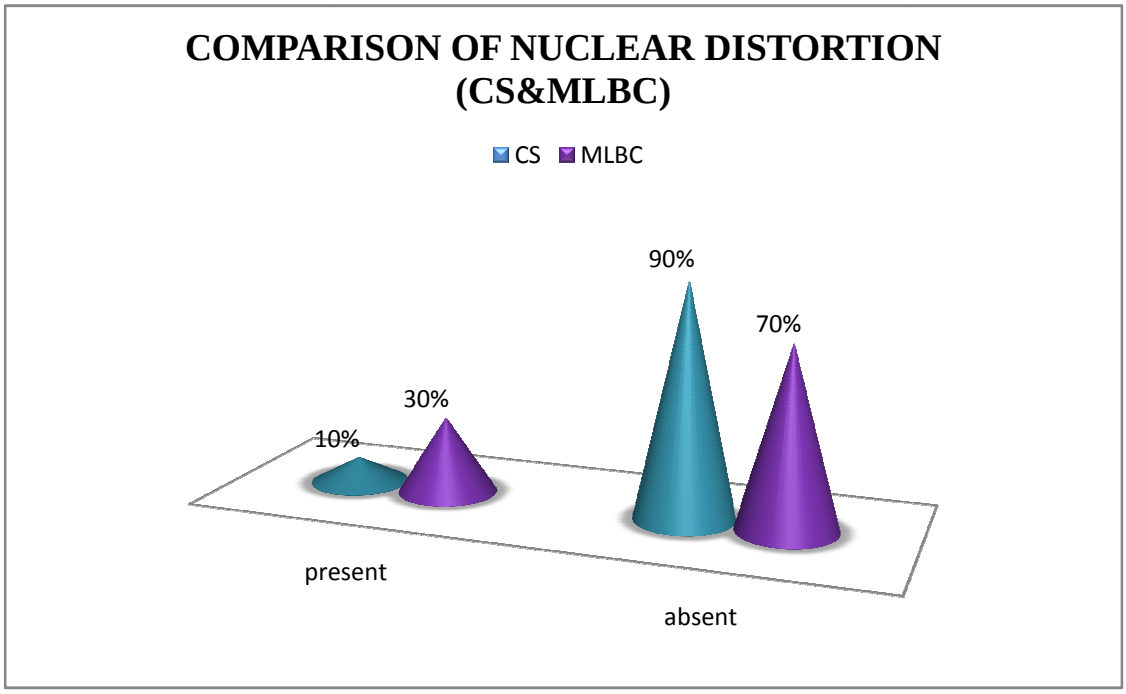
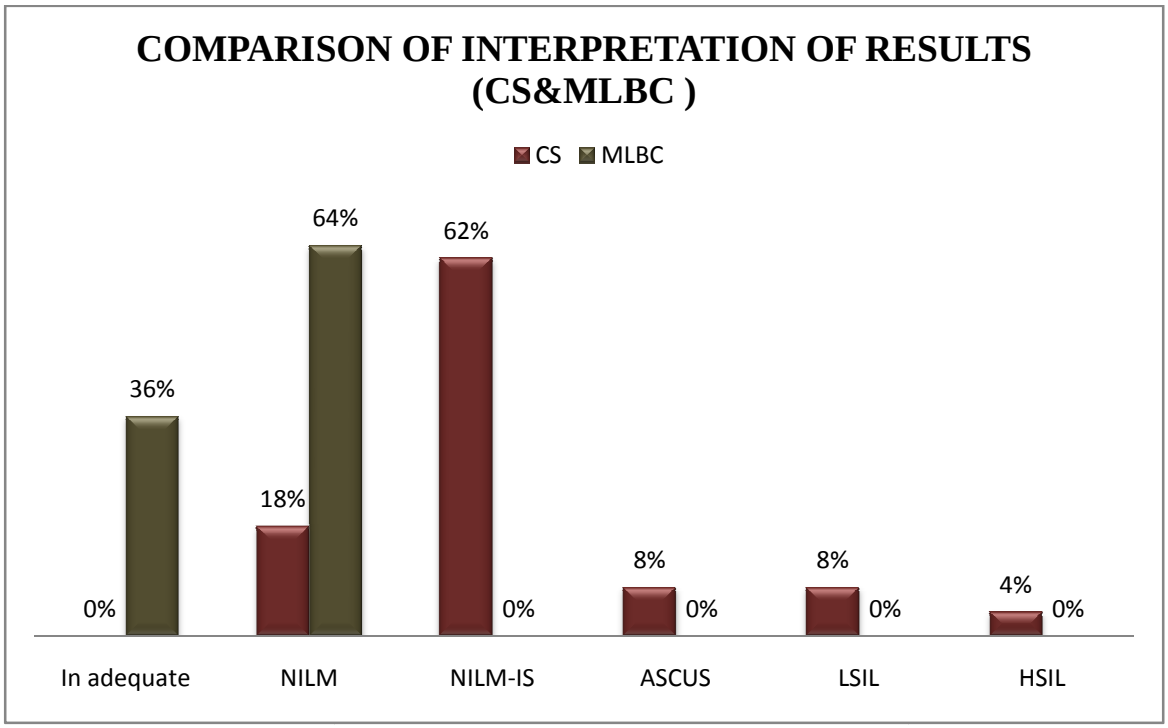


CHART 9



II. STATISTICAL ANALYSIS OF LP &CS RESULTS

Another group of 50 cytology smears were split and compared by CS &LP method. The data's are analyzed & interpreted by χ^2 (Chi-square) test by the statistical software IBM SPSS statistics 20. The P-values <0.05 (P<0.05) were treated as statistically significant.

TABLE-19:COMPARISON OF CELLULARITY (CS vs LP)

CELLULARITY	CS	LP	TOTAL	x^2	DF	SIGNIFICANCE
Adequate	44(88%)	47(94%)	91	1.099	1	P>0.05
Inadequate	6(12%)	3(6%)	9			
Total	50	50	100			

Of the 50 cases adequate cellularity was found in 47cases (94%) of LP smears & 44 cases (88%) of CS .The results revealed that there was no significant difference in cell adequacy between the two procedures (P>0.05). **(TABLE 19 & CHART 10).**

CHART 10

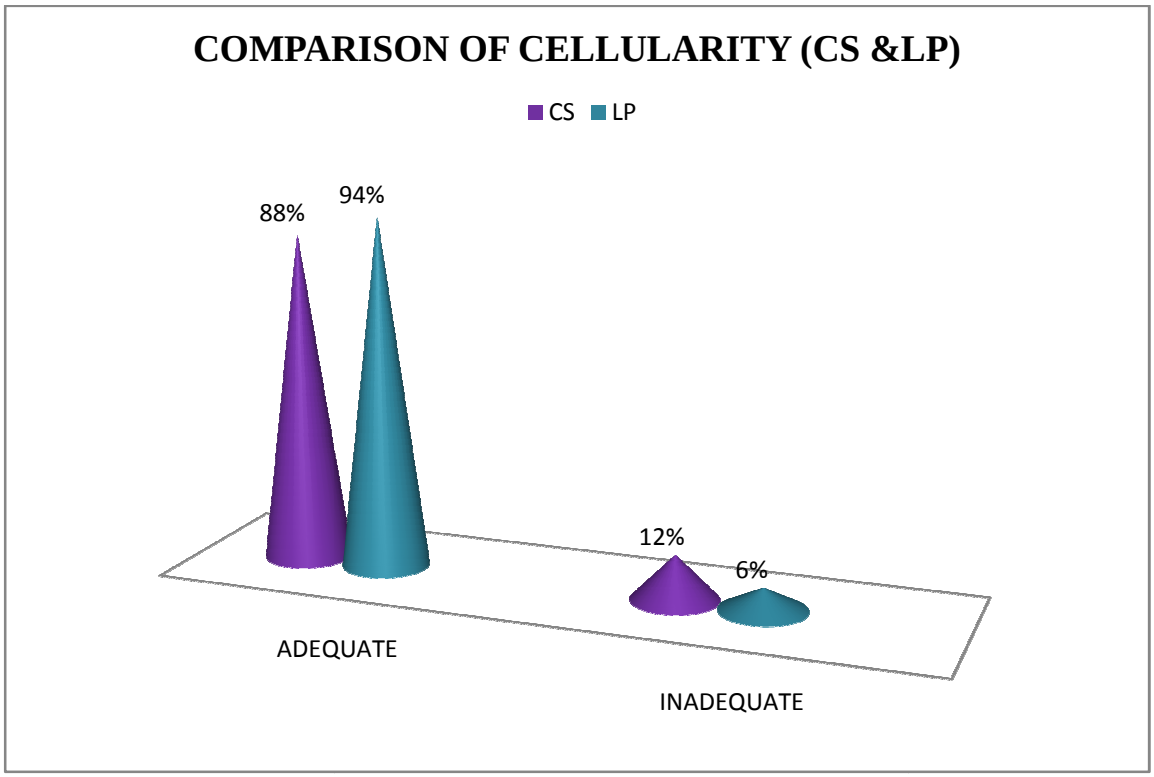

COMPARISON OF CELLULARITY (CS &LP)
CS
LP
88%
94%
12%
6%
ADEQUATE
INADEQUATE

TABLE- 20: COMPARISON OF CLEAN BACKGROUND

CLEAN BACK GROUND	CS	LP	TOTAL	x^2	DF	SIGNIFICANCE
Present	0(0%)	36(72%)	36	56.250	1	P<0.001
Absent	50(100%)	14(28%)	64			
Total	50	50	100			

Of 50 cases clean background was seen in 36(72%) cases of LP preparations but none of the CS showed clean background. The results revealed that there was statistically significant difference between the two procedures (P<0.001).**(TABLE 20 &CHART 11).**

TABLE-21:COMPARISON OF UNIFORM DISTRIBUTION OF CELLS

UNIFORM DISTRIBUTION	CS	LP	TOTAL	x^2	DF	SIGNIFICANCE
Present	4(8%)	30(60%)	34	30.125	1	P<0.001
Absent	46(92%)	20(40%)	66			
Total	50	50	100			

Uniform distributions of cells were found in 30 (60%) cases of LP smears whereas it was observed in only 4 (8%) cases of CS. This shows significant statistical difference between two methods (P<0.001). **(TABLE 21 &CHART 12).**

CHART 11

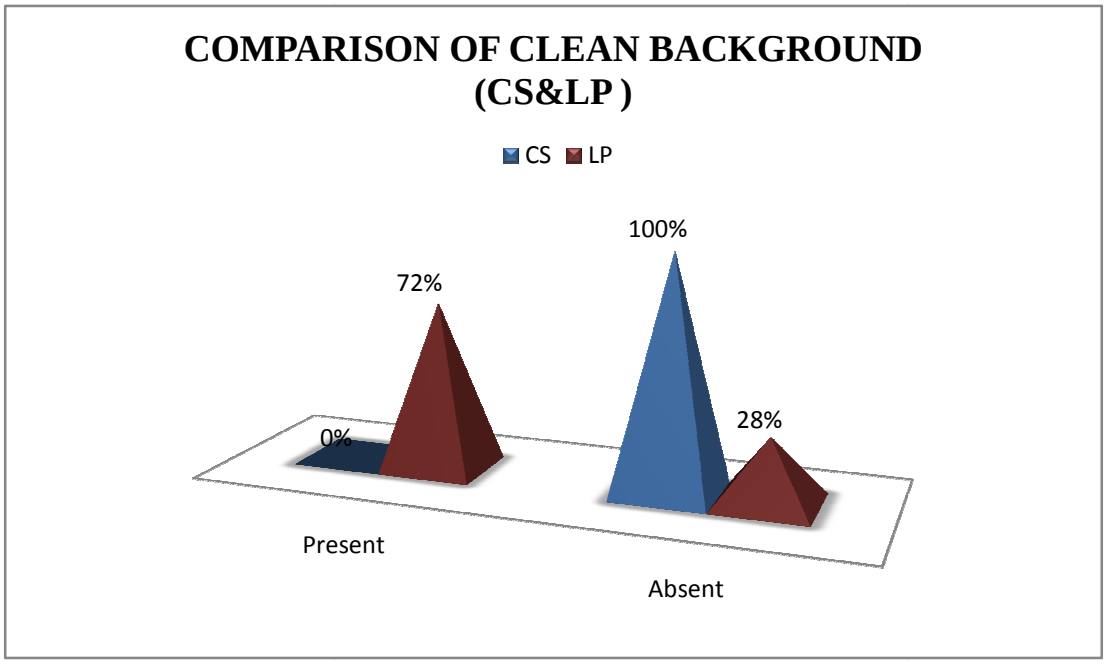


CHART 12

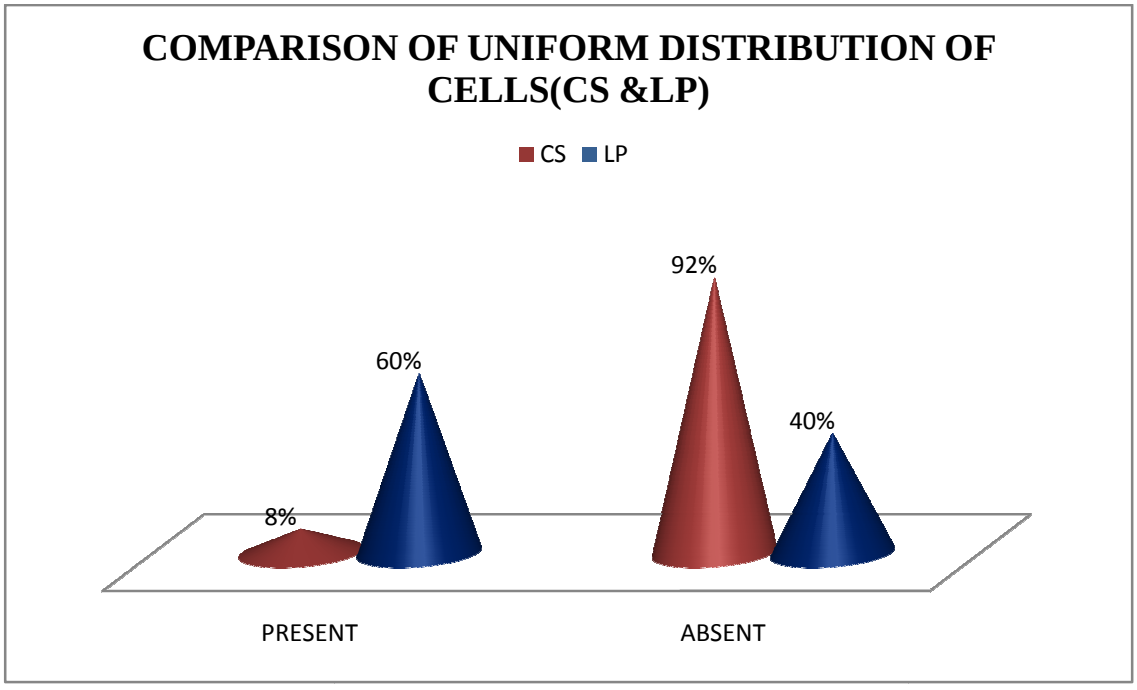


TABLE-22.COMPARISON OF CELL OVERLAPPING

CELL OVERLAPPING	CS	LP	TOTAL	x^2	DF	SIGNIFICANCE
Present	47(94%)	19(38%)	66	34.98	1	P<0.001
Absent	3(6%)	31(62%)	34			
Total	50	50	100			

Of 50 cases cellular overlapping was seen in 19(38%) cases of LP preparations and 47(94%) cases of CS .The results revealed that cell overlapping was seen more in CS, which was statistically very significant (P<0.001).**(TABLE 22& CHART 13).**

TABLE- 23. COMPARISON OF INFLAMMATORY CELLS IN THE BACKGROUND

INFLAMMATORY BACKGROUND	CS	LP	TOTAL	x^2	DF	SIGNIFICANCE
Present	18(36%)	9(18%)	27	4.110	1	P<0.05
Absent	32(64%)	41(82%)	73			
Total	50	50	100			

Of 50 cases inflammatory back ground was seen in 9 (18%) cases of LP preparations & 18(36%)cases of CS. The results revealed that the inflammatory back ground in CS was statistically differed with the LP procedure (P<0.05). **(TABLE 23 &CHART 14).**

CHART 13

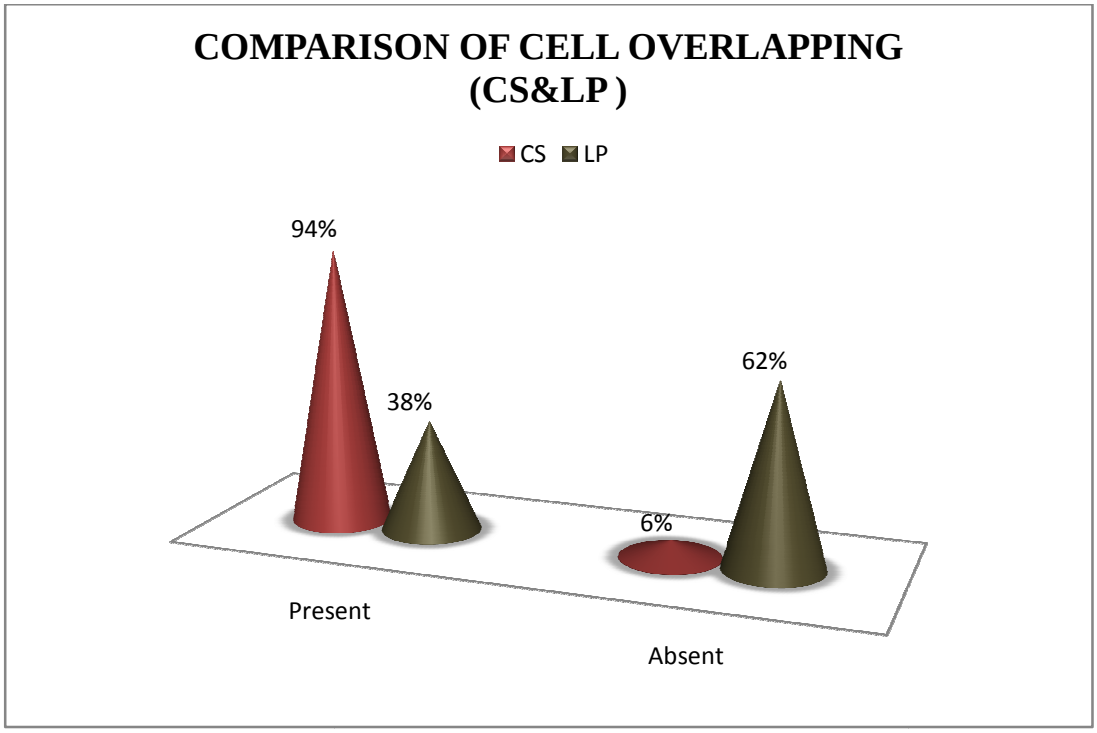


CHART 14

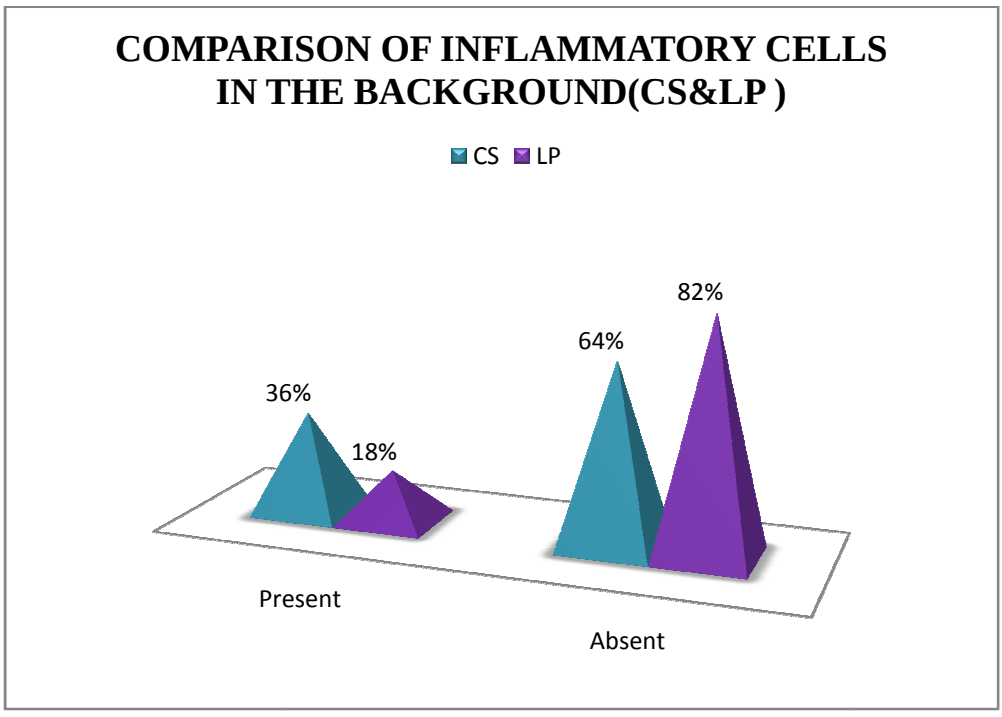


TABLE-24.COMPARISON OF CYTOPLASMIC DISTORTION

CYTOPLASMIC DISTORTION	CS	LP	TOTAL	x^2	DF	SIGNIFICANCE
Present	13(26%)	14(28%)	27	0.822	1	P>0.05
Absent	37(74%)	36(72%)	73			
Total	50	50	100			

Cytoplasmic distortion was seen in 14 (28%) & 13 (26%) cases of LP and CS slides respectively, which was not statistically significant (P>0.05). **(TABLE 24 &CHART 15).**

TABLE-25.COMPARISON OF NUCLEAR DISTORTION

NUCLEAR DISTORTION	CS	LP	TOTAL	x^2	DF	SIGNIFICANCE
Present	19(38%)	19(38%)	38	0.000	1	P=1.00
Absent	31(62%)	31(62%)	62			
Total	50	50	100			

The nuclear distortion in both procedures was equal(19 cases-38%) (P=1.00**).(TABLE 25 &CHART 16).**

CHART 15

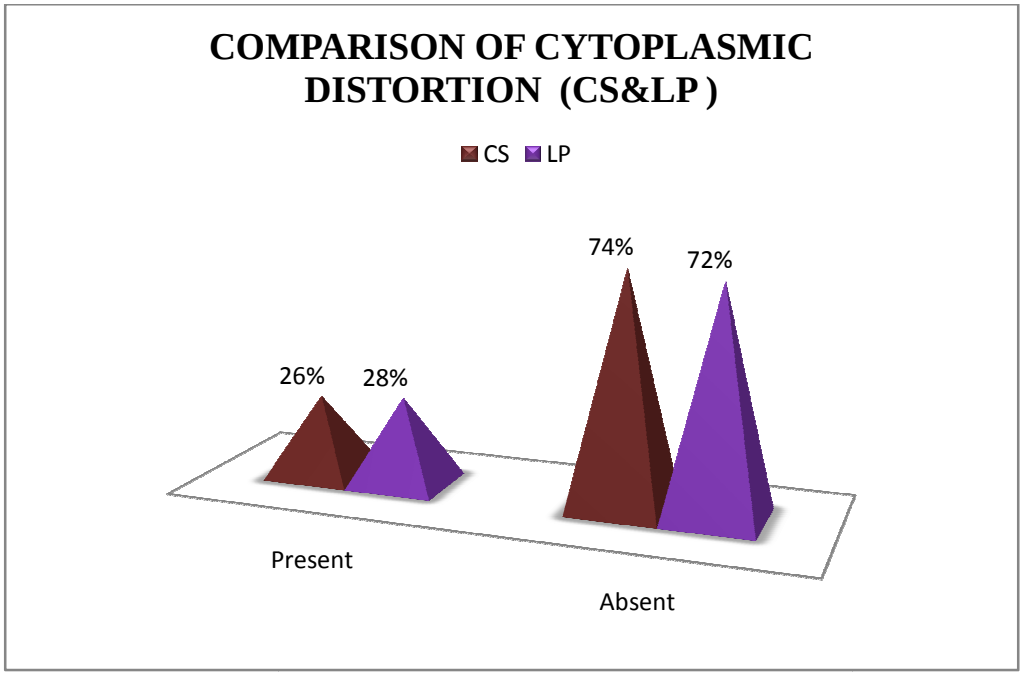


CHART 16

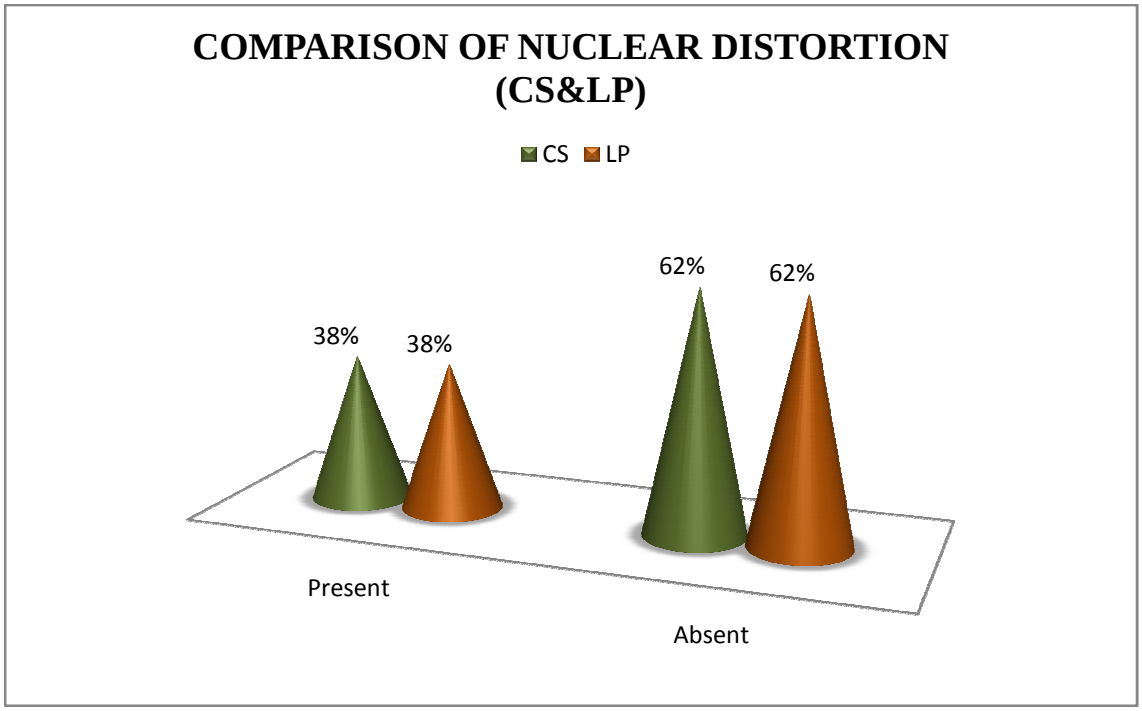
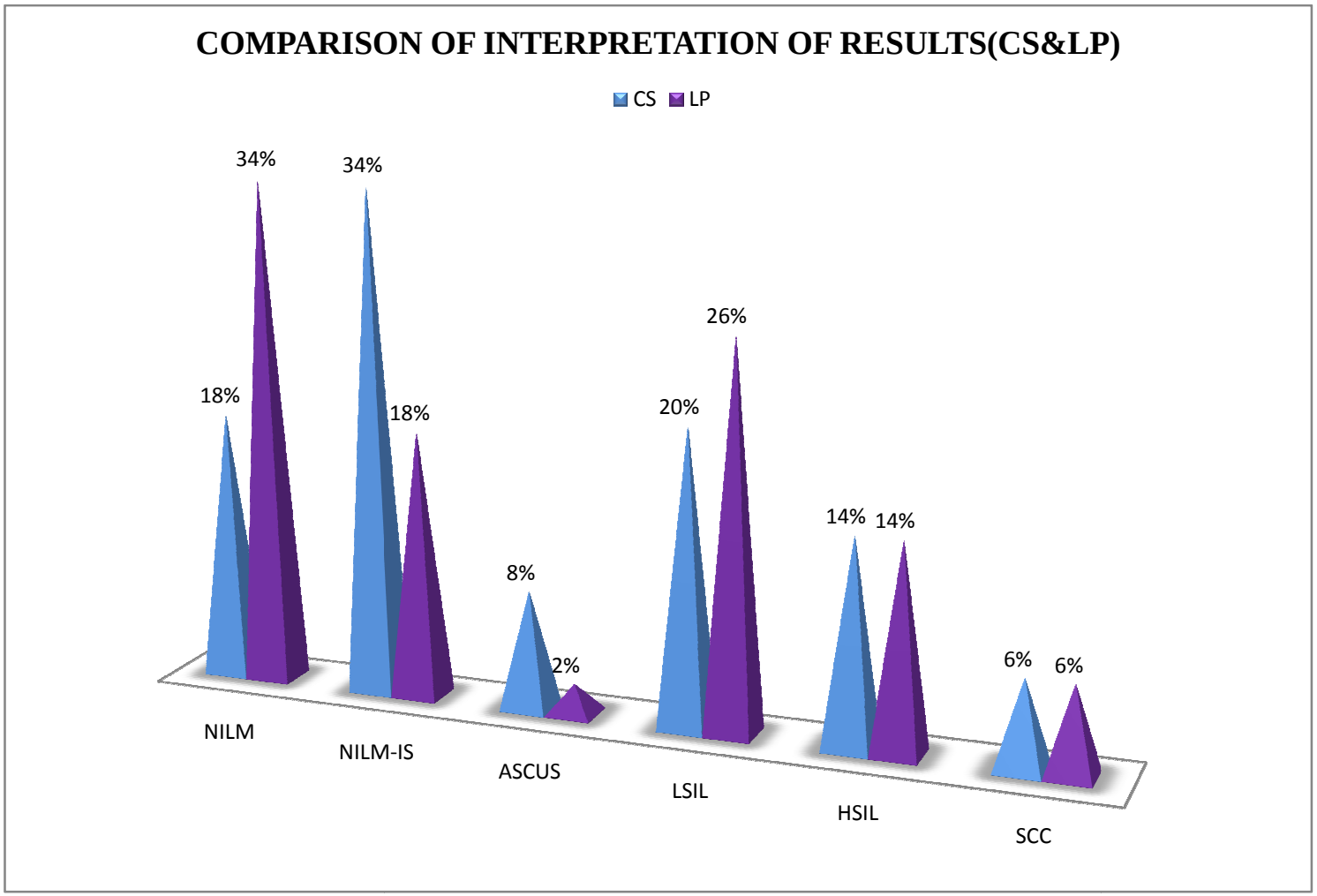


TABLE-26. INTERPRETATION OF RESULTS OF CS versus LP.

INTERPRETATION	CS	LP	TOTAL	x^2	DF	SIGNIFICANCE
NILM	9(18%)	17(34%)	26	7.114	5	P>0.05
NILM-IS	17(34%)	9(18%)	26			
ASCUS	4(8%)	1(2%)	5			
LSIL	10(20%)	13(26%)	23			
HSIL	7(14%)	7(14%)	14			
SCC	3(6%)	3(6%)	6			
Total	50	50	100			

In LP method 17 (34%) of cases were reported as NILM & 9(18%) of cases were reported as NILM-IS. In CS this was 9(18%) &17(34%) respectively.10 (20%) & 13(26%) of cases reported as LSIL by CS &LP method respectively. 7(14%) of cases were reported as HSIL &3(6%) of cases as SCC cases in both methods. Interpretation of results by the two procedures were not statistically significantly (P>0.05). **(TABLE 26 &CHART 17).**

CHART 17


COMPARISON OF INTERPRETATION OF RESULTS(CS&LP)
CS
LP
34%
34%
18%
18%
8%
2%
20%
26%
14%
14%
6%
6%
NILM
NILM-IS
ASCUS
LSIL
HSIL
SCC

III.STATISTICAL COMPARISON BETWEEN MLBC &LP METHOD (NON CASE MATCHED)

The MLBC and LP groups were compared on all 7 morphologic feature .The data of MLBC and LP were compared between them by Z test of proportions.

TABLE 27. COMPARISON OF MLBC VS LP

CATEGORY	RESULTS	MLBC N=50		LP N=50		Z	P
		No	%	No	%		
Cellularity	Inadequate	18	36	3	6	3.683	P<0.001
	Adequate	32	64	47	94		
Clean background	Present	29	58	36	72	1.484	P>0.05
	Absent	21	42	14	28		
Uniform distribution	Present	12	24	30	60	3.916	P<0.001
	Absent	38	76	20	40		
Cell overlapping	Present	33	66	19	38	2.919	P <0.01
	Absent	17	34	31	62		
Inflammatory background	Present	0	0.0	9	18	3.313	P<.0.001
	Absent	50	100.0	41	82		
Cytoplasmic distortion	Present	24	48	14	28	2.105	P<.0.05
	Absent	26	52	36	72		
Nuclear distortion	Present	15	30	19	38	0.847	P>0.05
	Absent	35	70	31	62		

Comparison of MLBC & LP method showed inadequate cellularity in 36% of cases of MLBC slides compared with 6 % in LP. i.e cellularity is significantly lower in MLBC. Clean background was seen in 29 (58%) cases of MLBC & 36 (72%) cases of LP which was not significantly different(($P>0.05$). The uniform distribution of cells are seen in 12(24%) & 30(60%) of cases of MLBC &LP method respectively. This was statistically significant($P<0.001$). Cell overlapping was observed in 33(66%) of MLBC cases & 19(38%) LP cases .This was statistically significant ($P <0.01$). The inflammatory cells were not present in MLBC group whereas it was present in 9(18%) cases of LP. Cytoplasmic distortion differed significantly and was seen more in MLBC (48%)($p<0.05$). Nuclear distortion did not significantly differ between MLBC & LP method (30%&38%) ($P>0.05$) . **(TABLE 27).**

CONVENTIONAL SMEAR

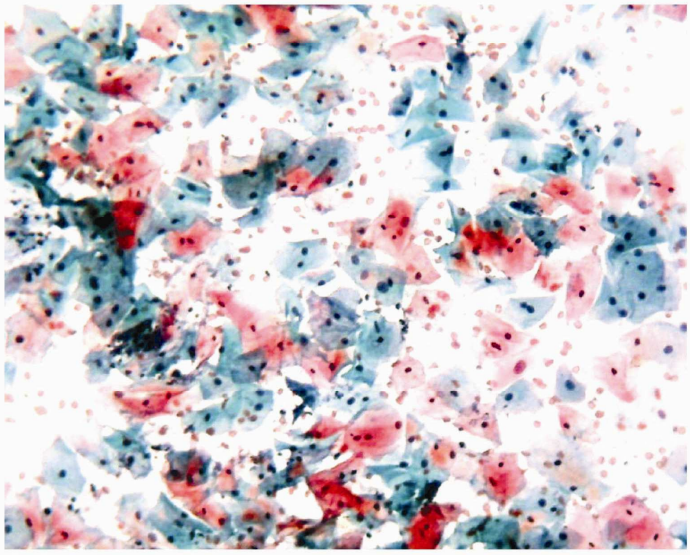


FIG 4: Cellular overlapping & inflammatory cells in the background (100X) PAP stain.

LIQUI PREP SMEAR

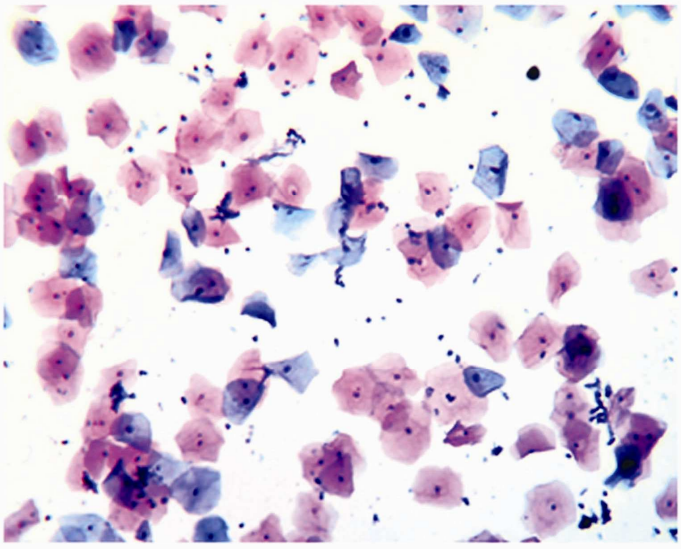


FIG 5: Uniform distribution of cells in a clean Background (100X) PAP stain.

CONVENTIONAL SMEAR

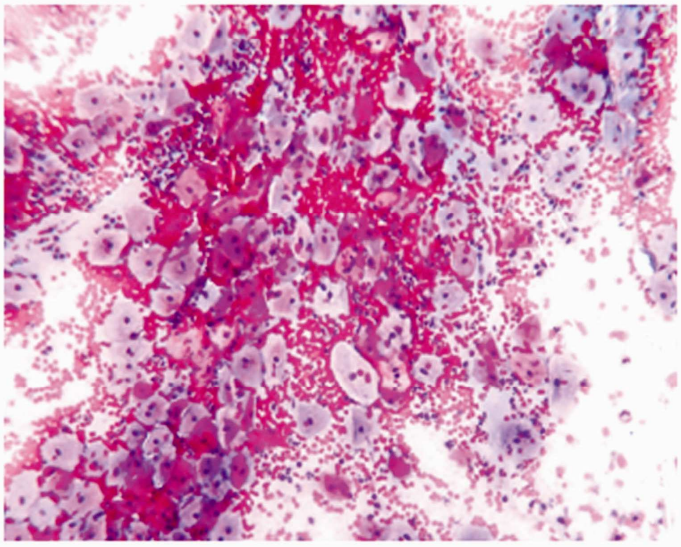


FIG 6: Hemorrhagic background. (100X)PAP stain.

LIQUI PREP SMEAR

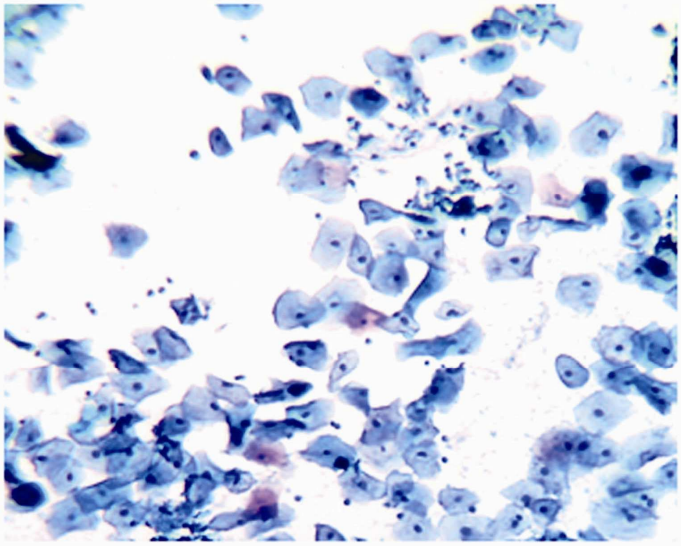


FIG 7: Absence of hemorrhagic background (100X)PAP stain.

CONVENTIONAL SMEAR

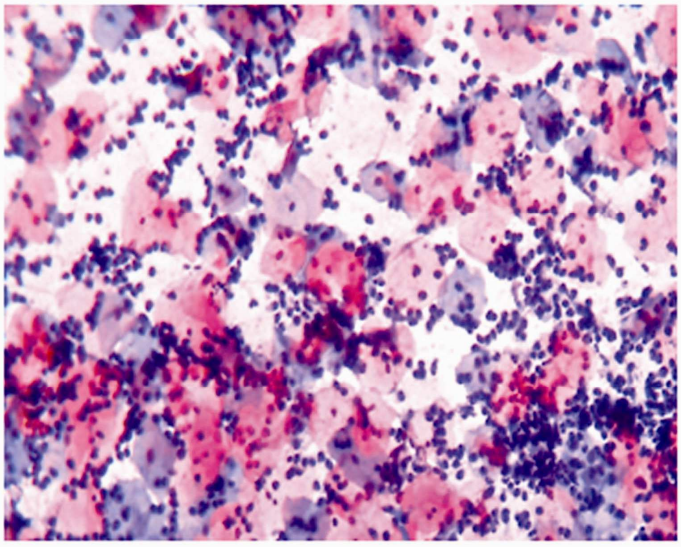


FIG 8: Abundant inflammatory cells in the background (100X)PAP stain

LIQUI PREP SMEAR

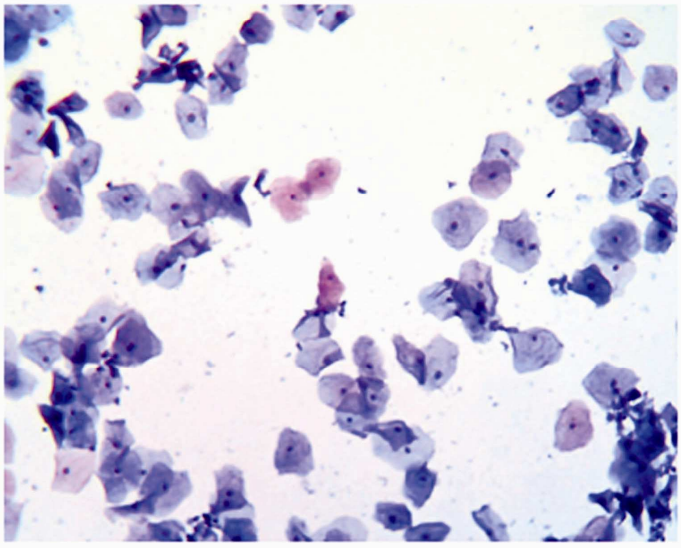


FIG 9:Absence of inflammatory cells in the background (100X)PAP stain.

CONVENTIONAL SMEAR

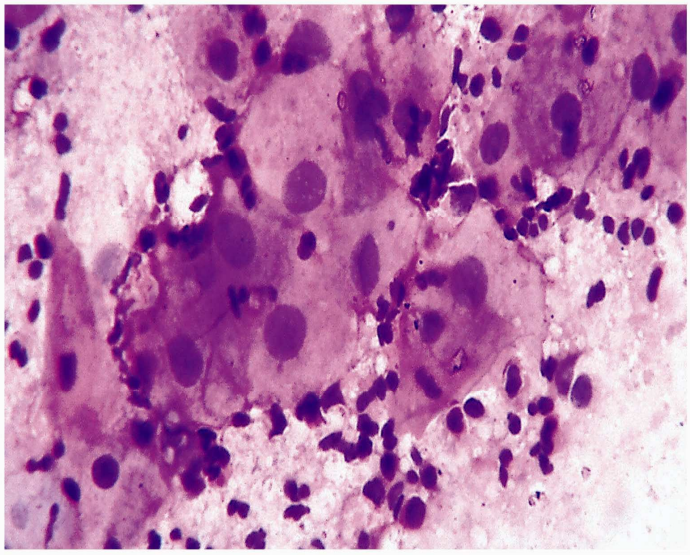


FIG 10: LSIL (400X)PAP stain.

LIQUI PREP SMEAR

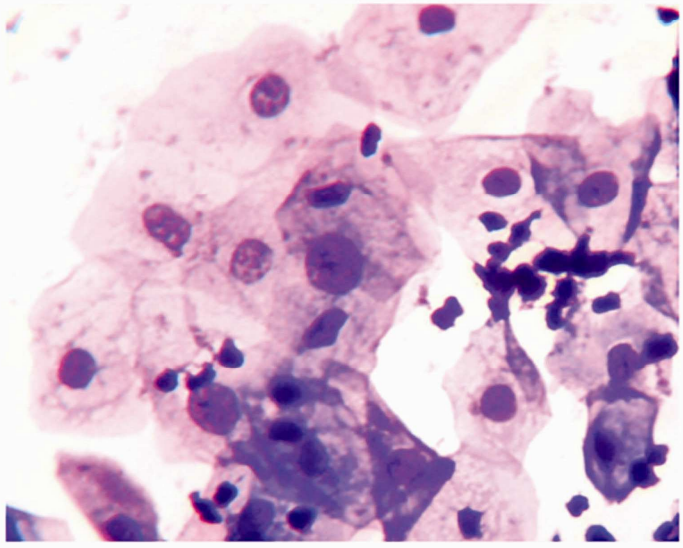


FIG 11: LSIL (400X)PAP stain.

CONVENTIONAL SMEAR

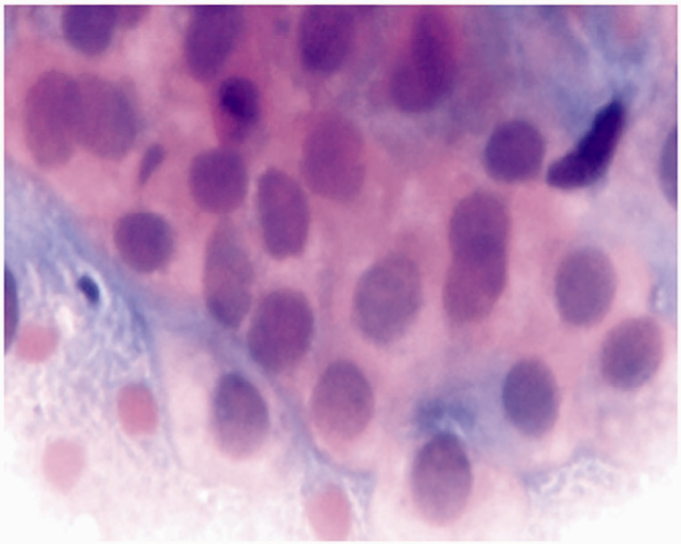


FIG 12: HSIL (400X)PAP stain.

LIQUI PREP SMEAR

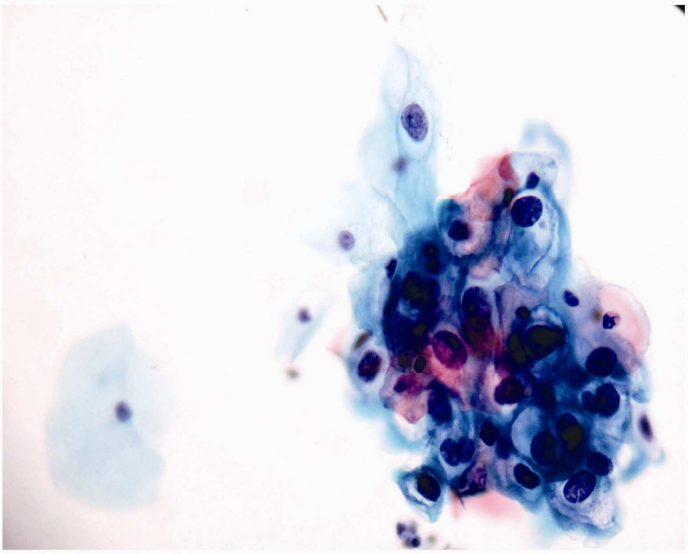


FIG 13: HSIL(400X)PAP stain.

CONVENTIONAL SMEAR

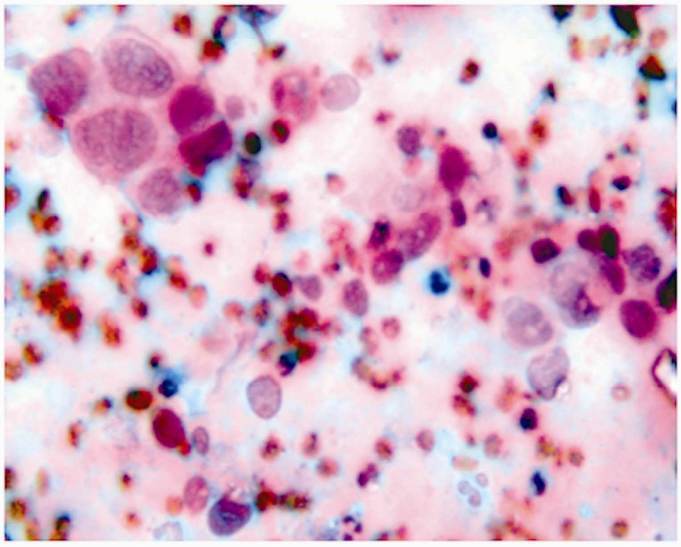


FIG 14: SCC (400X) PAP stain.

LIQUI PREP SMEAR

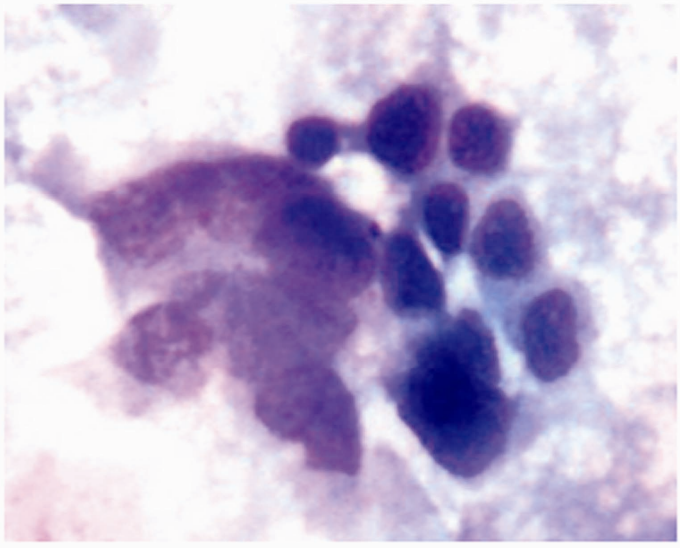


FIG 15: SCC(400X)PAP stain.

CONVENTIONAL SMEAR

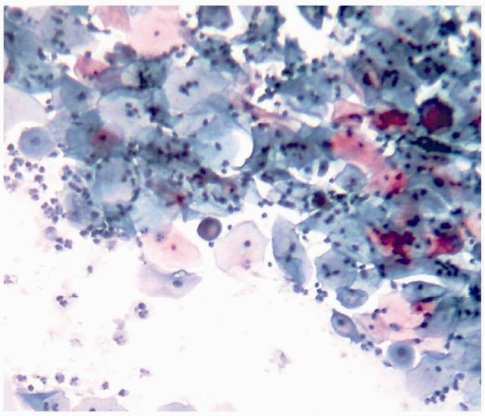


FIG 16:Cellular overlapping & excessive inflammatory cells in the background (100X) PAP stain.

MLBC

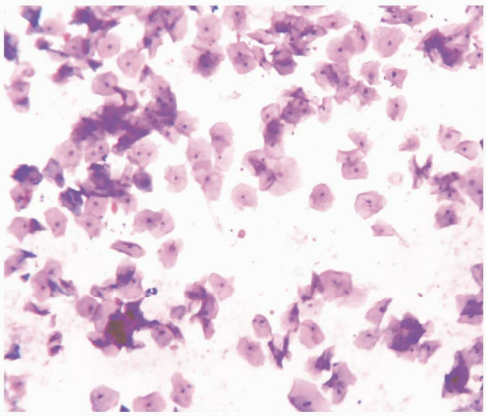


FIG 17: Reduced Cellular over-lapping & inflammatory cells in the background(100X)PAP stain.

MLBC

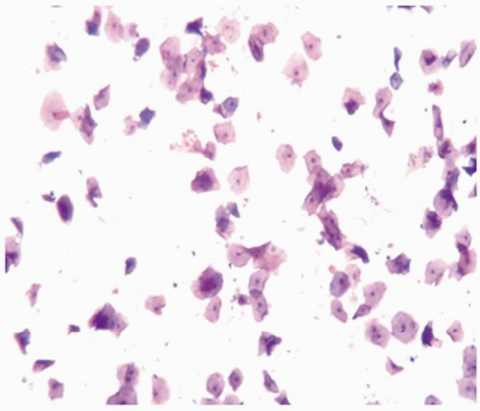


FIG 18:uniform distribution of cells (100X) PAP stain

DISCUSSION

The pap smear is a commonly used screening test for cervical cancer detection. First generation automated Liquid based cytology improves the quality of cervical smears through an improved way of slide preparation following collection of samples in a standard way. It provides more representative sample of specimen with reduced obscuring background material which allows faster and more reliable screening .The cost of this test is high, but there is increase in detection of pre invasive lesions and decrease in the number of indeterminate results such as ASC (Limaye et al 2003) [73](Trench 2000) [74]. In addition ancillary studies such as HPV testing can be done on residual sample (Levi et al 2003) [75].

A newer second generation technique of LBC-LiquiPrep has been developed to address the cost limitation of this automated method. This new second generation LP does not require any special equipments. It can be used as an alternative to CS in developing countries as there will be the advantages of LBC without its high cost. Recently another indigenous method of LBC called Manual Liquid Based Cytology(MLBC) has been studied in which preservatives and polymer solutions prepared in one's own laboratory has been used for processing cervical smears(Maksem et al 2001) [17](Maksem et al

2005) [18] (Lee et al 2006) [19] (Kavatkar et al 2008) [20] (Nandini et al 2012) [21].

In this study we compared Cervical smears prepared by this new Manual Liquid-based cytology with the conventional pap smears. In addition we also compared second generation LP smears with conventional smears. The smears are compared on the morphological parameters such as cellular adequacy, clean background, uniform distribution, cell overlapping, cytoplasmic distortion , nuclear distortion, inflammatory background and finally interpretation of results was done based on The Bethesda System 2001.

I.MANUAL LIQUID BASED CYTOLOGY (MLBC) versus CONVENTIONAL SMEARS(CS)

Manual Liquid Based Cytology (MLBC) is a technique that enables cells to be suspended in a monolayer sheet and thus improves detection of precursor lesions .There are only a few studies which have dealt with manual liquid based cytology and of these only some studies have compared MLBC with conventional smears. For MLBC method we prepared a fixative & cell base in our laboratory. We standardized this solution with 50 cases. Other 50 cases were compared with CS for various morphological features and final interpretation.

In MLBC preparations intact membrane of polymer solution that hold the cells to the slide indicate good processing technique.A study conducted by Kavatkar et al (2008) [20] showed intact membrane in 97(92%)cases out of 105 cases. In our study, intact membrane was observed in 64% of cases.

A study conducted by NM Nandini et al (2012) [21] showed more number of satisfactory smears in MLBC (99%) method than CS (91%). A study by Kavatkar et al (2008) [20] showed that MLBC based preparations were satisfactory in 92% cases compared to 90% of cases in CS. In contrast to these studies our study showed satisfactory (adequate cellularity) smear in more number of cases of CS than MLBC. In MLBC most of the unsatisfactory smears are due to scant cellularity in our study.

A study conducted by NM Nandini et al (2012)[21] showed clean background in all cases of MLBC which was not the case with their CS. This correlate with our study that also showed clean background in more cases of MLBC smears (58%) compared to CS(6%).

A study by NM Nandini et al (2012)[21]showed uniform distribution of cells in most of the cases of MLBC compared to CS. In our study uniform distribution of cells were found in 24% of MLBC preparations and 14% of conventional smears.

The studies conducted by Maksem et al (2001) [17] & Kavatkar et al (2008) [20] showed cellular overlapping in most of the CS compared to MLBC preparations . This is in concurrence with our study which showed cellular overlapping in more number of CS (86%) than MLBC preparations(66%).

A study conducted by Kavatkar et al(2008)[20]showed inflammatory cells in MLBC smears but without overlying the epithelial cells. Another study by NM Nandini et al (2012) [21]showed that inflammatory infiltrates were observed in less number of MLBC (20%) cases compared to CS (42%)cases. In our study Inflammatory cells were not seen in any case, as the inflammatory cells were removed in 100% of MLBC smears whereas 68% of CS showed inflammatory background.

In our study cytoplasmic distortion was found to be high in MLBC (48%) than CS (12%).The nuclear distortion was also high in MLBC smears (30%) compared to CS (10%).

A study by NM Nandini et al (2012)[21]on comparing the interpretation of smears showed same number of normal smears in both methods. But diagnosis of Low grade squamous intraepithelial lesion (36%) was more by MLBC method.

A study by Kavatkar et al (2008)[20] showed that there was an 88.8% agreement in the diagnosis by both method.2 cases of HSIL on

CS was reported as unsatisfactory on MLBC &1 case of ASCUS on MLBC was reported as NILM on CS in our study.

In our study 36%(18) of cases were reported as inadequate smears in MLBC ,due to scant cellularity and 64% (32 cases) were reported as Negative for intraepithelial lesions or malignancy(NILM). Two cases of NILM on MLBC were reported as HSIL in CS. These slides on CS showed predominantly normal squamous cells with few clusters showing high grade intraepithelial lesions. Two cases of inadequate smear and 2 cases of NILM on MLBC were interpreted as LSIL in CS. 3 cases of inadequate smears and one case of NILM on MLBC were reported as ASCUS on CS. This revealed that there was a statistically significant difference in the interpretation of results between the two methods . In our study MLBC smears are not able to pick up these cases with intraepithelial lesions, mostly due to less cellularity.

The study by Mc Googan et al (1998) [76] revealed that the effectiveness of LBC depends on proper collection and smearing method. Austin et al (1998) [46] found endocervical components more in CS than MLBC, which has been attributed to the split sample collection protocol and this can be overcome by direct sampling method.

We followed split sample method in our study to get slides of same patient for both MLBC and CS method, that provide us case

matched slides but which could have probably been a limiting factor for MLBC, as we didn't pick up intraepithelial lesions in 10 cases. Direct sampling method for MLBC could have detected more intraepithelial lesions.

TABLE 28:SUMMARY OF COMPARISON OF MLBC AND CONVENTIONAL PAP SMEARS ON MORPHOLOGICAL FEATURES IN OUR STUDY

MORPHOLOGICAL FEATURES	MLBC	CS
Cellularity	Adequate (64%)	Adequate (100%)
Clean background	Present (58%)	Present (6%)
Uniform distribution	Present (24%)	Present (14%)
Cellular overlapping	Present (66%)	Present (86%)
Cytoplasmic distortion	Present (48%)	Present (12%)
Nuclear distortion	Present (30%)	Present (10%)
Inflammatory cells	Absent (100%)	Present (68%)

In our study MLBC method was found to be comparable to the conventional pap smear in some parameters and inferior to CS in others. MLBC method provides more number of smears with clean background, uniform distribution without cell overlapping. Inflammatory

cells in the background are also completely removed. However, cytoplasmic & nuclear distortion was found to be higher in MLBC slides.

II.LIQUI PREP (LP) versus CONVENTIONAL SMEAR (CS)

In this study we also compared split samples of cervical smears by second generation LiquiPrep and conventional smear cytology.

TABLE:29 COMPARISON OF CELLULARITY IN VARIOUS STUDIES

STUDIES	ADEQUATE CELLULARITY (SATISFACTORY SMEAR)	
	LP	**CS**
1.**OUR STUDY**	**94%**	**88%**
2. Nadereh Behtash et al 2008	94.7%	92.1%
3.Jongkolnee Settakorn et al2008	94.1%	99.87%
4. M Tunc Canda et al 2009	98.3%	95.1%
5.Mahmood Khaniki et al 2009	94.7%	92.1%
6. Deshou et al 2009	More cellular	Less cellular

The studies conducted by Nadereh Behtash et al (2008) [77] Mahmood Khaniki et al (2009) [58] Deshou et al (2009) [56]&M Tunc Canda et al (2009) [59] showed adequate cellularity in more number of LP preparations than CS. This correlate with our study as it also showed adequate cellularity in more number of LP (94%) cases compared to CS (88%).In contrast , a study conducted by Jongkolnee Settakorn et al(2008) [16] showed more number of satisfactory smears in CS (99.87%)

than LP (94.1%) .Whereas the study conducted by Davey et al (2006)[78] showed no difference in the rate of detection of satisfactory slides by both the methods. Our study shows that LP method provide more number of adequate smear than CS.

A study conducted by Deshou et al (2009)[56] showed clean background in majority of LP preparations. This correlate with our study as it also showed clean background in 72% of LP samples. Whereas clean background was not observed in any conventional smear preparations.

A study by Deshou et al (2009)[56] showed uniform distribution of cells commonly in CS but not in LP. This is in contrast to our study that showed uniform distribution of cells in more cases of LP (60%) preparations than CS (8%).

A study conducted by Deshou et al (2009)[56]showed cellular overlapping in all cases of CS but only in two cases of LP (0.006%).This is in concurrence with our study which also showed cellular overlapping in more number of CS (94%) than the LP preparations (38%)

A study conducted by M Tunc Canda et al (2009)[59] showed a considerable reduction in inflammatory cells in LP smears. Our study also showed that inflammatory cells obscuring the epithelial cell morphology was observed in more number of CS (36%) than LP (18%) preparations.

A study by Deshou et al (2009) [56] showed cellular morphological changes in most of CS samples but rarely in LP samples. Our study showed cytoplasmic distortion in 28% of LP smears and 26% of CS. Nuclear distortion was found to be same (38%) in both the methods .

The study conducted by Mahmood Khaniki et al (2009) [58] showed more number of ASCUS cases in CS(1.43%) than in LP(0.79%). A study by Park et al (2007) [61] showed that the rate of detection of ASCUS was more in LP(6.5%) than the CS(2.8%). In our study ASCUS was reported more in CS (8%) than LP (2%) smears. This correlate with the study by Mahmood Khaniki et al. This shows that LP method reduces the number of indeterminate results such as ASC.

In the present study LP method detected more number of LSIL (26%) cases compared to CS (20%). The rate of HSIL (14%) and SCC (6%) was equal in both methods.

Deshou et al (2009) [56]& M Tunc Canda et al (2009) [59] showed LP resulted in remarkable increase in detection rate of ASCUS, ASC-H, AGC, LSIL, HSIL and SCC over Conventional smears. Our study correlates with all the above studies and showed that LiquiPrep method is a reliable method of cervical cytology screening.

TABLE :30 SUMMARY OF COMPARISON OF LP AND CONVENTIONAL PAP SMEARS ON MORPHOLOGICAL FEATURES IN OUR STUDY

MORPHOLOGICAL FEATURES	LP	CS
Cellularity	Adequate (94%)	Adequate (88%)
Clean background	Present (72%)	Absent (100%)
Uniform distribution	Present (60%)	Present (6%)
Cellular overlapping	Present (38%)	Present (94%)
Cytoplasmic distortion	Present (28%)	Present (26%)
Nuclear distortion	Present (38%)	Present (38%)
Inflammatory cells	Present (18%)	Present (36%)

In our study a significant number of LP smears showed adequate cellularity,clean background ,uniform cell distribution without overlapping and reduction in inflammatory cells in the background compared to CS. However no significant difference in the number of cases with cytoplasmic or nuclear distortion were noted between two methods. LP method reduces the number of inflammatory smears and thereby provide a better morphological evaluation of epithelial cells. The LP method also showed an increased rate of detection of LSIL cases compared to CS and reduces the rate of indeterminate results such as ASCUS smears .

III. COMPARISION OF MANUAL LIQUID BASED CYTOLOGY WITH LIQUIPREP METHOD

In our study morphological parameters were compared between the two LBC methods.

TABLE:31 SUMMARY OF COMPARISON OF MLBC WITH LP IN OUR STUDY

MORPHOLOGICAL FEATURES	MLBC	LP
Cellular adequacy	Adequate (64%)	Adequate (94%)
Clean background	Present (58%)	Present (72%)
Uniform distribution	Present (24%)	Present (60%)
Cellular overlapping	Present (66%)	Present (38%)
Cytoplasmic distortion	Present (24%)	Present (28%)
Nuclear distortion	Present (30%)	Present (38%)
Inflammatory cells	Absent (100%)	Present (18%)

On comparison of MLBC & LP method we found that LP method provide more number of adequately cellular smears compared to MLBC. Clean background & uniform distribution was also seen more commonly with LP procedure. In addition LP method showed reduced cellular overlapping thereby allowing better morphological assessment of cells. Cytoplasmic features are not much altered in both methods. The MLBC preparations were entirely devoid of inflammatory cells in the

background but in case of LP, inflammatory cells were seen in 18% of cases. MLBC preparations failed to detect all the cases of intraepithelial squamous cell abnormality. This has been due to reduced cellularity in our MLBC slides. All intraepithelial lesions were detected when the slides were processed by LP method. The rate of indeterminate results such as ASCUS was lower in LP smears than MLBC smears.

SUMMARY & CONCLUSION

This study was conducted to Evaluate Second generation Liquid Based Cytology (LiquiPrep) and Manual Liquid Based Cytology and to compare it with conventional cervical smears.

Conventional cervical cytology is a simple, cost effective method of cervical cancer screening that has been in use for more than 50 years and is still a highly effective screening procedure. It is widely used because of easy method of preparation of slides and interpretation of results. In our study most of the Conventional preparations showed cellular overlapping, inflammatory cells, blood and mucus that obscure the epithelial cell morphology which was much reduced in LP &MLBC. Inspite of this conventional cytology is a sensitive method of cervical screening and it detected all cases of intraepithelial lesions in our study.

Comparison of morphological details and results of cervical cytology smears by all the three methods showed that LiquiPrep method provides more representative sample with reduced obscuring material which allows better morphological evaluation. LP method also generated higher number of satisfactory smears compared to conventional smears & MLBC. In addition LP method detects more cases of intraepithelial lesions when compared to MLBC.

Manual Liquid Based Cytology method provides cytology smears with clean background that do not have inflammatory cells in any of the

slides. But still the percentage of satisfactory smears is less compared to LP& CS .All the intraepithelial lesions of cervix could not be detected by our MLBC method. This has been due to reduced cellularity in some cases or non representation of abnormal cells in other cases.

Our study highlights that LiquiPrep method provides better cytomorphological features compared to Conventional smear and Manual LBC. Manual Liquid Based Cytology method has to be improved with more standardization to increase the cellularity and more representation of abnormal cells at par with LiquiPrep, if it has to replace the low cost Conventional smear in developing countries.

COMPARISON OF CONVENTIONAL SMEARS AND LIQUI PREP SMEARS

S.NO	AGE	OP .NO	CELLULARITY		CLEAN BACKGROUND		UNIFORM DISTRIBUTION		CELL OVERLAPPING		INFLAMMATORY BACKGROUND		CYTOPLASMIC DISTORTION		NUCLEAR DISTORTION		INTERPRETATION	
			CS	LP	CS	LP	CS	LP	CS	LP	CS	LP	CS	LP	CS	LP	CS	LP
1	35	29060	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	NILM-IS	NILM-IS
2	36	29090	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
3	25	29200	Inadequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
4	60	29024	adequate	Adequate	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present	Present	Present	HSIL	HSIL
5	34	29883	Adequate	Adequate	Absent	Present	Absent	Absent	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
6	30	29091	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	NILM	NILM
7	42	29094	Inadequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	NILM	NILM
8	32	29096	Adequate	Adequate	Absent	Present	Present	Present	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent	NILM-IS	NILM-IS
9	25	29655	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
10	50	39811	Adequate	Adequate	Absent	Present	Absent	Absent	Present	Present	Present	Present	Present	Present	Absent	Absent	NILM-IS	NILM-IS
11	34	40909	Adequate	Adequate	Absent	Present	Absent	Absent	Present	Present	Present	Present	Absent	Absent	Absent	Absent	NILM-IS	NILM-IS
12	54	29905	Adequate	Adequate	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Present	Present	Present	Present	HSIL	HSIL
13	44	29865	Adequate	Adequate	Absent	Present	Absent	Absent	Present	Present	Present	Absent	Present	Present	Absent	Absent	NILM-IS	NILM
14	37	29940	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	NILM-IS	NILM-IS
15	32	30030	Adequate	Adequate	Absent	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Present	Present	ASCUS	ASCUS
16	42	30071	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Present	Present	LSIL	LSIL
17	48	30075	Adequate	Adequate	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Present	Present	Present	Present	LSIL	LSIL
18	22	30056	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	NILM	NILM
19	42	29950	Adequate	Adequate	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Present	Present	Present	Present	SCC	SCC
20	35	28788	Adequate	Adequate	Absent	Present	Absent	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	Absent	LSIL	LSIL
21	56	33012	Adequate	Adequate	Absent	Present	Absent	Absent	Present	Present	Absent	Absent	Present	Present	Present	Present	SCC	SCC
22	42	40672	Inadequate	Inadequate	Absent	Absent	Absent	Absent	Present	Present	Present	Absent	Present	Absent	Absent	Absent	NILM-IS	NILM
23	60	40776	Adequate	Adequate	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Present	Present	Present	Present	SCC	SCC
24	48	40777	Adequate	Adequate	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Present	Present	Present	Present	HSIL	HSIL
25	27	40902	Inadequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	NILM	NILM
26	45	40915	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Present	Present	LSIL	LSIL
27	40	40906	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	NILM-IS	NILM-IS
28	30	40907	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Present	Present	ASCUS	LSIL
29	40	40984	Adequate	Adequate	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Present	Present	Present	Present	HSIL	HSIL
30	42	40938	Adequate	Adequate	Absent	Present	Present	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	NILM	NILM
31	47	40893	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	NILM	NILM
32	55	45118	Adequate	Adequate	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Absent	Present	Present	Present	HSIL	HSIL
33	29	41197	Adequate	Adequate	Absent	Present	Absent	Present	Present	Present	Absent	Absent	Present	Present	Present	Present	ASCUS	LSIL
34	50	40736	Adequate	Adequate	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Absent	Present	Present	Present	LSIL	LSIL
35	35	41605	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
36	62	41748	Adequate	Adequate	Absent	Present	Absent	Present	Present	Present	Present	Present	Absent	Absent	Absent	Absent	NILM-IS	NILM-IS
37	35	41736	Adequate	Adequate	Absent	Absent	Absent	Present	Present	Present	Absent	Absent	Absent	Present	Absent	Absent	LSIL	LSIL
38	35	67738	Inadequate	Adequate	Absent	Present	Absent	Absent	Present	Absent	Absent	Absent	Present	Absent	Absent	Absent	NILM	NILM
39	40	49593	Adequate	Adequate	Absent	Present	Absent	Absent	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	LSIL	LSIL
40	29	67651	Adequate	Inadequate	Absent	Present	Absent	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	NILM-IS	NILM-IS
41	35	47651	Adequate	Adequate	Absent	Present	Absent	Absent	Present	Absent	Absent	Absent	Absent	Absent	Present	Present	ASCUS	LSIL
42	22	41924	Adequate	Inadequate	Absent	Present	Absent	Absent	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
43	29	41946	Adequate	Adequate	Absent	Absent	Absent	Present	Present	Present	Present	Present	Absent	Absent	Absent	Absent	NILM -IS	NILM-IS
44	18	41118	Adequate	Adequate	Absent	Present	Present	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	NILM	NILM
45	40	41980	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Present	Present	LSIL	LSIL
46	40	41269	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Present	Present	HSIL	HSIL
47	37	42037	Inadequate	adequate	Absent	Absent	Present	Present	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	NILM	NILM
48	28	41267	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Present	Absent	Absent	Absent	Present	Present	HSIL	HSIL
49	48	41567	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	LSIL	LSIL
50	48	42367	Adequate	Adequate	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	LSIL	LSIL

COMPARISON OF CONVENTIONAL SMEARS AND MANUAL LIQUID BASED CYTOLOGY SMEARS

S.NO	AGE	OP .NO	CELLULARITY		CLEAN BACKGROUND		UNIFORM DISTRIBUTION		CELL OVERLAPPING		INFLAMMATORY BACKGROUND		CYTOPLASMIC DISTORTION		NUCLEAR DISTORSION		INTERPRETATION	
			CS	MLBC	CS	MLBC	CS	MLBC	CS	MLBC	CS	MLBC	CS	MLBC	CS	MLBC	CS	MLBC
1	60	200769	3	1	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Present	Present	Present	Present	LSIL	INADEQUATE SMEAR
2	45	212926	3	2	Absent	Present	Absent	Present	Present	Absent	Present	Absent	Present	Absent	Absent	Absent	NILM-IS	NILM
3	42	212931	3	2	Absent	Present	Present	Present	Absent	Absent	Present	Absent	Absent	Present	Absent	Absent	NILM-IS	NILM
4	39	212989	3	2	Absent	Present	Absent	Absent	Present	Present	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
5	50	212996	3	2	Absent	Present	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent	Present	Present	HSIL	NILM
6	58	195548	3	2	Absent	Absent	Absent	Present	Present	Absent	Present	Absent	Absent	Present	Present	Present	ASCUS	NILM
7	35	54265	3	2	Absent	Present	Absent	Absent	Present	Present	Present	Absent	Absent	Present	Absent	Present	NILM-IS	NILM
8	32	204877	3	2	Absent	Absent	Present	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
9	55	143234	3	2	Absent	Present	Present	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	Absent	NILM	NILM
10	50	21684	3	1	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Absent	Present	Absent	Present	NILM	INADEQUATE SMEAR
11	28	113796	2	2	Absent	Present	Absent	Absent	Present	Present	Absent	Absent	Present	Present	Absent	Absent	NILM	NILM
12	50	227863	3	3	Absent	Present	Present	Present	Absent	Absent	Present	Absent	Present	Absent	Absent	Present	NILM-IS	NILM
13	48	233013	3	1	Absent	Absent	Absent	Absent	Present	Present	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	INADEQUATE SMEAR
14	34	233252	3	2	Absent	Present	Absent	Absent	Absent	Absent	Present	Absent	Absent	Absent	Absent	Present	NILM-IS	NILM
15	25	233188	3	1	Absent	Absent	Absent	Absent	Present	Present	Present	Absent	Absent	Present	Absent	Present	NILM-IS	INADEQUATE SMEAR
16	59	235076	3	3	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Present	Present	HSIL	NILM
17	27	233693	3	3	Absent	Present	Absent	Present	Present	Absent	Present	Absent	Present	Absent	Absent	Present	NILM-IS	NILM
18	47	224276	3	2	Absent	Present	Absent	Present	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
19	45	233673	3	1	Absent	Absent	Absent	Absent	Present	Absent	Present	Absent	Absent	Present	Absent	Present	NILM-IS	INADEQUATE SMEAR
20	35	6829	3	1	Absent	Absent	Absent	Absent	Present	Absent	Present	Absent	Absent	Present	Absent	Absent	ASCUS	INADEQUATE SMEAR
21	39	6848	3	2	Absent	Present	Absent	Absent	Present	Present	Present	Absent	Absent	Absent	Absent	Present	NILM-IS	NILM
22	34	6853	3	3	Absent	Absent	Absent	Present	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
23	42	6841	3	1	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Absent	Present	Present	Present	LSIL	INADEQUATE SMEAR
24	33	10468	3	1	Present	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Present	Absent	Absent	NILM	INADEQUATE SMEAR
25	39	10034	3	1	Absent	Absent	Absent	Absent	Present	Present	Present	Absent	Absent	Present	Absent	Absent	NILM-IS	INADEQUATE SMEAR
26	30	20111	3	1	Present	Absent	Absent	Absent	Absent	Present	Absent	Absent	Absent	Present	Absent	Absent	ASCUS	INADEQUATE SMEAR
27	38	10521	3	2	Absent	Present	Absent	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
28	46	20210	2	2	Absent	Present	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	NILM	NILM
29	38	13502	3	1	Absent	Present	Absent	Absent	Present	Present	Absent	Absent	Absent	Present	Absent	Absent	NILM	INADEQUATE SMEAR
30	25	54051	3	2	Absent	Present	Present	Absent	Present	Present	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
31	25	54009	3	2	Absent	Present	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	LSIL	NILM
32	48	13540	3	1	Absent	Absent	Absent	Absent	Present	Present	Present	Absent	Absent	Present	Absent	Absent	NILM-IS	INADEQUATE SMEAR
33	28	13499	3	2	Absent	Present	Absent	Absent	Present	Present	Present	Absent	Present	Absent	Absent	Absent	NILM-IS	NILM
34	24	13530	3	1	Absent	Absent	Absent	Absent	Present	Present	Present	Absent	Absent	Present	Absent	Absent	NILM-IS	INADEQUATE SMEAR
35	22	31197	3	2	Absent	Present	Absent	Absent	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
36	50	20736	3	1	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Absent	Present	Absent	Absent	NILM	INADEQUATE SMEAR
37	35	11605	3	2	Absent	Present	Absent	Absent	Present	Present	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
38	62	21748	2	2	Absent	Present	Absent	Absent	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
39	35	41736	2	3	Absent	Present	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	NILM	NILM
40	35	37738	3	2	Absent	Present	Absent	Absent	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	NILM-IS	NILM
41	40	29593	3	3	Present	Present	Absent	Present	Present	Absent	Present	Absent	Absent	Absent	Absent	Present	NILM-IS	NILM
42	29	27651	2	3	Absent	Present	Absent	Present	Present	Absent	Present	Absent	Absent	Present	Absent	Absent	NILM-IS	NILM
43	32	28456	3	1	Absent	Absent	Absent	Absent	Present	Present	Present	Absent	Absent	Present	Absent	Absent	ASCUS	INADEQUATE SMEAR
44	31	32637	3	2	Absent	Present	Present	Absent	Present	Present	Present	Absent	Absent	Present	Absent	Absent	NILM-IS	NILM
45	37	27034	3	2	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Present	LSIL	NILM
46	35	28493	2	1	Absent	Absent	Absent	Absent	Present	Present	Present	Absent	Absent	Present	Absent	Absent	NILM-IS	INADEQUATE SMEAR
47	38	20036	2	1	Absent	Absent	Absent	Absent	Present	Present	Present	Absent	Absent	Present	Absent	Absent	NILM-IS	INADEQUATE SMEAR
48	40	21675	2	1	Absent	Absent	Absent	Absent	Present	Present	Present	Absent	Absent	Present	Absent	Absent	NILM-IS	INADEQUATE SMEAR
49	35	28360	3	2	Absent	Present	Absent	Absent	Present	Present	Present	Absent	Absent	Present	Absent	Absent	NILM-IS	NILM
50	49	27890	2	2	Absent	Present	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	NILM	NILM

BIBLIOGRAPHY

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics .CA Cancer J Clin. Mar-Apr 2011;61 (2):69-90.
2. Ferlay J, Shin H, Bray F, et al. Estimates of worldwide burden of cancer in: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-917.
3. Clarke EA, Anderson TW. Does screening by Pap smears help prevent cervical cancer?.A case-control study.Lancet.1979;2:1-4.
4. Hakama M et al. Evaluation of screening programmes for gynaecological cancer. British Journal of Cancer1985; 52: 669-673.
5. Miller AB et al. Report on a workshop of the UICC project on evaluation of screening for cancer. International Journal of Cancer 1990; 46:761-769
6. Mathew A, George PS. Trends in incidence and mortality rates of squamous cell carcinoma and adenocarcinoma of cervix – worldwide. Asian Pac J Cancer Prev 2009;10:645-650.
7. Wright T, et al: Precancerous lesions of the cervix. In Kurman R(ed): Blaustein's Pathology of Female Genital Tract,4th ed. New York, Springer 1994;p 229.
8. Papanicolaou GN ,Traut HF. The diagnostic value of vaginal smears in carcinoma of the uterus. Am J Obstet Gynecol 1941; 42:193-206.
9. Fahey MT, Irwig L, Macaskill P. Meta-analysis of Pap test accuracy. American Journal of Epidemiology 1995; 141:680-689.

10. Nanda K et al. Accuracy of the Papanicolau test in screening for and follow-up of cervical cytologic abnormalities: A systematic review. Annals of Internal Medicine 2000; 132:810-819.
11. Richart RM, Valliant HW. Influence of cell collection techniques upon cytologic diagnosis. Cancer 1965; 11: 1474-1478.
12. Lee KR, Ashfaq R, Bidrsong GG, Corkill ME, McIntosh KM, Inhorn SL. Comparison conventional papanicolaou smears and a fluid-based,thin-layer system for cervical cancer screening. Obstet Gynecol 1997;90(2):278-84.
13. Monsonego J, Utillo-Touati A, Bergeron C, Dachez R, Liaras J, Saurel J ,et al. Liquid-based cytology for primary cervical screening: a multi-centre study.Br J Cancer 2001;84(3):360-6.
14. Fang-Hui Zhao, Shang-Ying Hu, Jessica J. Bian. Comparison of ThinPrep and SurePath Liquid-Based Cytology and Subsequent Human Papilloma virus DNA Testing in China. Cancer Cytopathol 2011;119:387–94.
15. Geyer J,Marino J, editors. Evaluation of Liquiprep encapsulation method for Liquid Based cytology: cell loss estimate during processing. Proceedings of the 15th International Congress of Cytology 2004 ;Santiago,Chile.Amsterdam:Elsevier;2004;p.176.
16. Settakorn J, Rangdaeng S, Preechapornkul N, et al . Inter observer reproducibility with LiquiPrep TM liquid based cervical cytology

screening in a developing country. Asian Pacific J Cancer Prev 2008; 9:92-6.

17. John A. Maksem ,Finnemore M, Belsheim BL.et al. Manual method for liquid based cytology .A demonstration using 1000 gynecological cytologies collected directly to vial and prepared by a smear –slide techniques. Diagn Cytopathol 2001;25:334-338.
18. John A. Maksem, Vijaya Dhanwada V, Joy E. Trueblood ,James Wiedmann ,Bruce Kane ,David R.Bolick,et al. Testing automated liquid based cytology samples with manual LBC method using residual cell suspension from 500 Thin Prep cases. Diagn cytopathology 2005;34(6):391-6
19. Lee, Kelly D, Gravitt PE, et a. Validation of a low cost, liquid based screening method of cervical intraepithelial neoplasia. Am J Obstet & Gynaecology, 2006; 195**:** 965-70.
20. Anita N. Kavatkar, C.A. Nagwanshi, S.M. Dabak .Study of manual method of liquid based cervical cytology. Indian Journal Of Pathology and Microbiology 2008 ;51:2190-94.
21. NM Nandini, SM Nandish, P Pallavi, SK Akshatha, AP Chandrashekhar, S Anjali, Murali Dhar. Manual Liquid Based Cytology in Primary Screening for Cervical Cancer - a Cost Effective Preposition for Scarce Resource Settings. Asian Pacific J Cancer Prev 2012;13:3645-3651.

22. World Health Organisation 2009 b and National Cancer Registry programme, Atlas Of Cancer in India.
23. ICMR 2004,Assessment of Burden of Non communicable diseases.
24. American Cancer Society. Cancer Facts & Figures 2012. Atlanta:American Cancer Society Surveillance Research;2012.
25. Thomison J, Thomas LK, Shroyer KR. Human papilloma virus: molecular and cytologic/histologic aspects related to cervical intraepithelial neoplasia and carcinoma. Hum Pathol 2008; 39:154-166.
26. Walboomers, J.M, M.V. Jacobs, M.M. Manos, F.X. Bosch, J.A. Kummer,et al. Human papilloma virus is a necessary cause of invasive cervical cancer world-wide. J. Pathol. 1999; 189: 12-19
27. Munoz, N., Bosch. FX, S. de Sanjose, R. Horrero, X. Castellague, et al. Epidemiological classification of human papilloma virus types associated with cervical cancer. N. Engl. J. Med. 2003; 348: 518- 527.
28. Lee KR, Minter LJ, Crum CP: Koilocytotic atypia in Papanicolaou smears: reproducibility and biopsy correlations. Cancer 1997; 81:10-15.
29. Schiffman M et al. Human Papilloma virus and Cervical cancer. Lancet 2007;370:890.
30. Fox H, Wells M.eds. Haines &Taylor's Obstetrical and Gynaecological Pathology, Anatomy of cervix &physiological

changes in cervical epithelium.4th ed .vol 1. Edinburgh: Churchill Livingstone; 1987:225-242.

31. Richart RM: Cervical intraepithelial neoplasia. Pathol Annu 1973; 8:301.
32. Boschaun H.W. Definition of a superficial cell. Acta Cytol 1958;2:52.
33. Bertalanffy .F.D. Aspects of cell formation and exfoliation related to cyto diagnosis. Acta Cytol 1963;7:362.
34. Gondos B,Marshall,D, Ostergar,D.R. Endocervical cells in cervical smears. Am. J . Obstet.Gynecol 1972;114:833-834.
35. Fetherston.W.C. Squamous neoplasia of vagina related to DES syndrome.Am.J.Obstet.Gynecol1975;122:176.
36. Liu.W.,et at.Normal exfoliation of endometrial cells in premenopausal women.Actal.Cytol1963;7:211.
37. Boschaun,H.W. Cytomorphology of normal endometrium. Acta.Cytol 1958;2:52.
38. Ducatman BS, Wang HH: The PAP smear: controversies in practice. London, Arnold, 2002.
39. Papanicolaou GN. A new procedure for staining vaginal smears.Science 1942;95:438–439.
40. Cramer DW. The role of cervical cytology in the declining morbidity and mortality of cervix cancer. Cancer 1974;34:2018–2027.

41. Coppleson LW, Brown B. Estimation of the screening error rate from the observed detection rate in repeated cervical cytology. Am J Obstet Gynecol 1974;119:753–958.

42. .Hutchinson M, Patten FW, Stelzer GT, Hurley AA, et al. Study of cell loss in the conventional Papanicolaou smear. Acta Cytol 1992;36:557.

43. Steven FS, Palcic B, Sin J, Desai M. A simple clinical method for the preparation of improved cervical smears – approximating to monolayers. Anticancer Res 1997;**17**:629–32.

44. Gay JD, Donaldson LD, Goellner JR. False-negative results in cervical cytologic studies. Acta Cytol 1985;29:1043–1046.

45. Goodman A, Hutchinson ML. Cell surplus on sampling devices after routine cervical cytologic smears. A study of residual cell populations. J Reprod Med 1996;41(4):239-41.

46. Austin RM, Ramzy I. Increased detection of epithelial cell abnormalities by liquid-based gynecologic cytology preparations: A review of accumulated data.Acta Cytol 1998;42:178-84.

47. Baker JJ. Conventional and liquid based cervico vaginal cytology. A comparison study with clinical and histologic Follow-up. Diagn Cytopathology 2002; 27: 185-8.

48. Yukihiro Kobayashi et al . Liquid-Based Thin-Layer Cytology Can Be Routinely Used in Samples Obtained via Fiberoptic Bronchoscope. Acta Cytologica 2011;55:69-78

49. Abulafia O, Pezzullo JC, Sherer DM .Performance of ThinPrep liquid-based cervical cytology in comparison with conventionally prepared Papanicolaou smears: a quantative survey. Gynecol Oncol2003; 90: 137-44.
50. Park IA, Lee SN, chow SW, Kim JW. comparing the accuracy of thin prep test and conventional pap smear on the basis of the histological diagnosis-a clinical study.Acta Cytol 2001;45:525-31.
51. Bernstein SJ, Sanchez-Ramos L et al. Liquid-based cervical cytology smear study and conventional Papanicolaou smears: A meta analysis of prospective studies comparing cytologic diagnosis and sample adequacy. Am J Obstet Gynecol 2001; 185:308-17.
52. Annie N. Y. Cheung, Elaine F. Szeto, Betty S. Y. Leung, Ui-Soon Khoo. Liquid-Based Cytology and Conventional Cervical Smears .A Comparison Study in an Asian Screening Population .Cancer Cytopathol 2003;99:331–5.
53. Colgan TJ, McLachlin CM, Cotterchio M, et al. Results of the implementation of liquid-based cytology-Surepath in the Ontario screening program. Cancer 2004; 102: 362- 67.
54. B. Kirschner, K. Simonsen and J. Junge. Comparison of conventional Papanicolaou smear and SurePath_liquid-based cytology in the Copenhagen population screening programme for cervical cancer. Cytopathology 2006; 17: 187–194.

55. Maurice Fremont-Smith , James Marino, B., Bryan Griffin, Lynn Spencer et al. Comparison of the SurePath TM liquid-based Papanicolaou smear with the conventional Papanicolaou smear in a multi-site direct-to-vial study. Cancer Cytopathol 2004;102(5): 269-279.

56. Hao Deshou, Wang Changhua, Li Qinyan ,Liu Wei, Fu Wen. Clinical utility of LIQUI-PERP cytology system for primary cervical cancer screening in a large urban hospital setting in China. Journal Of Cytology 2009; 26:20-25.

57. Roghaei MA, Afshar Moghaddam N, Pooladkhan Sh. Adequacy Criteria and Cytomorphological Changes in Liqui-Prep TM versus Conventional Cervical Cytology.Shira E-Medical Journal 2010;11(4).

58. Mahmood Khaniki et al. compare the screening performance of a new modified liquid-based cytology method (Liquiprep™) with conventional PAP smear (CP) in a low risk population, using colposcopy followed by histology as “gold standard 2009;4(2):59-64.

59. M Tunc Canda, Namik Demir, Orcun Sezer, Latife Doganay, Ragip Ortac. Clinical Results of the Liquid-based Cervical Cytology Tool, Liqui-PREP TM, in Comparison with Conventional Smears for Detection of Squamous Cell Abnormalities. Asian Pacific J Cancer Prev, 2009;10:399-402.

60. Alives V.A, Bibbo M, Schmitt F.C. 2004.Comparision of manual and automated method of LBC.A morphological study. Acta Cytol,48: 187-93.

61. Joonseok Park, Eun-Ha Jung, Changok ,Young Hee . Direct-to-Vial comparison of a new Liquid –based cytology system , Liqui-PREP versus the conventional smears. Diagn Cytopathol 2007;35.8:488-92.

62. Schiffman M, Castle PE. The promise of global cervical-cancer prevention. N Engl J Med 2005;353:2101-4.

63. Solomon D, Nayar R. The Bethesda System for reporting cervical cytology.Definitions, Criteria and Explanatory notes.2nd ed. Heidelberg: Springer 2004.

64. George G. Birdsong , Diane D Davey, Teresa M. Darragh, et al . The Bethesda System for Reporting Cervical Cytology. Definitions, Criteria and Explanatory Notes. 2nd ed. Heidelberg: Springer 2004 .

65. Sherman ME, Solomon D, Schiffman M. Qualification of ASCUS .A comparison of equivocal LSIL and equivocal HSIL cervical cytology in ACSUS LSIL triage study. Am J Clin Pathol.2001;116:386-394.

66. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA. 2002;287:2114–2119

67. Clark SB, Dawson AE. Invasive squamous –cell carcinoma in ThinPrep specimen: diagnostic clues in the cellular pattern. Diagn Cytopathol 2002 ;26:1-4.

68. Renshaw AA, Young NA, Colgan TJ, et al .Comparison of performance of conventional and ThinPrep gynecological preparations in the college of American Pathologists gynecologic cytology program. Arch Pathol Lab Med 2004;128:17-22

69. Bollmann M, Bankfalvi A, Trosic A, Speich N, Schmitt C, Bollmann R. Can we detect cervical human papilloma virus (HPV) infection by cytomorphology alone? Diagnostic value of non-classic cytological signs of HPV effect in minimally abnormal Pap test. Cytopathology 2005; 16: 13–21.

70. Seybolt JF ,Johnson WD. Cervical cytodiagnostic problems .A survey. Am J Obstet Gynecol 1971;109:1089-1103.

71. Riotten G, Christopherson WM, Lunt R. Cytology of the Female Genital Tract (International Histological Classification of Tumors No. 8). Geneva: World Health Organization; 1973.

72. Mali SN, Wilkinson EJ, Drew PA ,et al. Benign cellular changes in Pap smears. Causes and significance. Acta Cytol 2001;45(1):5-8.

73. Limaye A, Connor A.J, Huang X. & Luff R. Comparative analysis of conventional Papanicolaou tests and a fluid-based thin-layer method. Arch Pathol Lab Med 2003; 127: 200-4.

74. Tench W. Preliminary assessment of the AutoCyte PREP. Direct-to-vial performance. J Reprod Med 2000; 45: 912-6.

75. Levi A.W., Kelly D.P., Rosenthal D.L. & Ronnett B.M. Atypical squamous cells of undetermined significance in liquid-based cytologic specimens: results of reflex human papillomavirus testing and histologic follow-up in routine practice with comparison of interpretive and probabilistic reporting methods. Cancer 2003; 99: 191-7.

76. McGoogan E, Colgan T, Remzy J, et al (1998). Cell preparation methods and criteria for sample adequacy. IAC Task Force summary. Acta Cytol, 42: 25-32.

77. Nadereh Behtash , Zeinb Nazari, Mahmod Khaniki, Kazem Zendedel et al. LiquiPrep a new liquid based cervical cytology method in comparison with conventional pap smear in developing countries. Research Journal of Biological Sciences 2008;3(6):627-630

78. Davey E, Barrat A, Irwing L, et al. Effect of study design and quality on unsatisfactory rates, cytology classifications, and accuracy in liquid-based versus conventional cytology: a systematic review. Lancet 2006; 367: 122-32.

APPENDIX 1

THE BETHESDA SYSTEM 2001

1.SPECIMEN TYPE:

Indicate conventional smear or Liquid Based Cytology or others.

2.SPECIMEN ADEQUACY

- Satisfactory for evaluation (note presence/absence of endocervical transformation zone component)
- Unsatisfactory for evaluation (specify reason)
    - ✓ Specimen rejected/not processed (specify reason)
    - ✓ Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason-obscuring blood,inflammation or scant cellularity)

3.GENERAL CATEGORIZATION (OPTIONAL)

- Negative for intraepithelial lesion or malignancy
- Epithelial cell abnormality
- Other

4.INTERPRETATION/RESULT

- Negative for intraepithelial lesion or malignancy
- **Organisms**
    - ✓ Trichomonas vaginalis
    - ✓ Fungal organisms morphologically consistent with Candida

- ✓ Shift in flora suggestive of bacterial vaginosis
- ✓ Bacteria morphologically consistent with Actinomyces
- ✓ Cellular changes consistent with herpes simplex virus

- **Other non-neoplastic findings (Optional to report)**
  - ✓ Reactive cellular changes associated with inflammation (includes typical repair), radiation intrauterine contraceptive device, Glandular cells status post hysterectomy, Atrophy

- **Epithelial cell abnormalities**
  - Squamous cell
    - ✓ Atypical squamous cell (ASC) of undetermined significance(ASCUS), cannot exclude HSIL (ASC-H)
    - ✓ Low-grade squamous intraepithelial lesion (LSIL)
    - ✓ High-grade squamous intraepithelial lesion (HSIL)
    - ✓ Squamous-cell carcinoma
  - **Glandular cell**
    - ✓ Atypical glandular cells (AGC) (specify endocervical, endometrial or not otherwise specified)
    - ✓ Atypical glandular cells, favour neoplastic (specify endocervical or not otherwise specified)
    - ✓ Endocervical adenocarcinoma in situ (AIS)
    - ✓ Adenocarcinoma

- **Other**
    - ✓ Endometrial cells in a woman ≥ 40 years of age.
- OTHER MALIGNANT NEOPLASM (specify)
- ANCILLARY TESTING
- AUTOMATED REVIEW
- EDUCATIONAL NOTES AND SUGGESTIONS(optional)

APPENDIX 2

PROFORMA

1 .PAP SMEAR NO : ..... 2. OP NO:.....

3. PATIENT NAME : .....

4. PATIENT AGE : .....

5 .ADDRESS : .....

.....

6.COMPLAINTS:

H/O Leucorrhea - Yes/No

H/O Inter menstrual Bleeding - Yes/No

H/O Postmenopausal Bleeding - Yes/No

7.MENSTRUAL H/O-LMP:

8.CLINICAL EXAMINATION:

Speculum Examination

9.ANALYSIS

FEATURES	CONVENTIONAL SMEAR	LBC
1.Sample Adequacy (Adequate/Not)		
2.Clean Background(+/-)		
3.Uniform Distribution(+/-)		
4.Cell Overlapping(+/-)		
5.Inflammatory cell Background(+/-)		
6.Cytoplasmic Distortion(+/-)		
7.Nuclear Distortion(+/-)		

10. INTERPRETATION OF RESULTS: